thermoscientific

NanoDrop Micro Volume UV/Vis Spectrophotometers and Fluorometers

# NanoDrop Ultra

## User Guide

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ThermoFisher SCIENTIFIC

For U.S. Technical Support, please contact:

Thermo Fisher Scientific
3411 Silverside Road
Tatnall Building, Suite 100
Wilmington, DE 19810 U.S.A.

Telephone: 302 479 7707
Toll Free: 1 877 724 7690 (U.S. & Canada only)
E-mail: nanodrop@thermofisher.com

For International Support, please visit:

http://www.thermofisher.com/NanoDropSupport

Contact your local distributor. For contact information go to:

http://www.thermofisher.com/NanoDropDistributors



For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.

**WARNING** Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

# Contents

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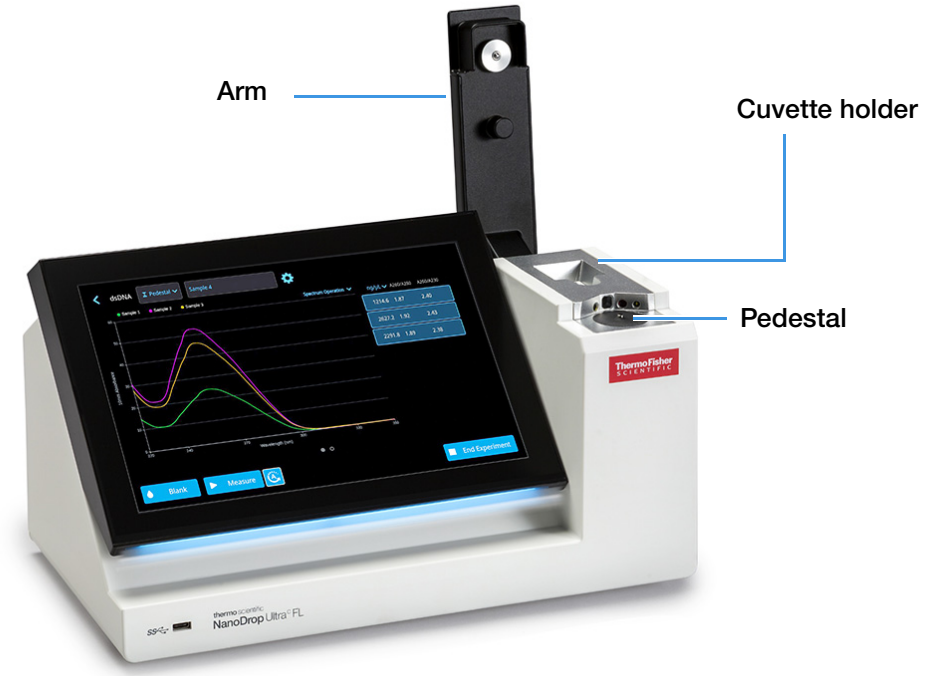
# About the Spectrophotometer

**NanoDrop Ultra$^{C}$ FL Spectrophotometer and Fluorometer**




**Note** Locate the instrument away from air vents and exhaust fans to minimize evaporation

The Thermo Scientific™ NanoDrop™ Ultra instrument series is composed of compact, stand-alone UV-Visible spectrophotometers and fluorometers developed for micro-volume analysis of a wide variety of analytes. The pioneering sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

The NanoDrop Ultra system comes with preloaded software and a touchscreen display. NanoDrop Ultra PC control software can be installed on a local PC and used to control the instrument and view data. The instrument can be connected to an optional printer with a USB cable or to a remote printer through an Ethernet connection or wireless network using the provided NanoDrop Ultra Wi-Fi and Bluetooth Dongle.

**Note** Before operating a NanoDrop Ultra instrument, please read the safety and operating precautions and then follow their recommendations when using the instrument.

# Features

The NanoDrop Ultra spectrophotometers and fluorometers feature the micro-volume sample retention system. The NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL models additionally feature a cuvette holder for analyzing dilute samples using standard UV-Visible cuvettes. The NanoDrop Ultra FL and NanoDrop Ultra$^{C}$ FL models are capable of both micro-volume absorbance and fluorescence measurements by including red and blue solid-state light emitting diodes (LED's), which are oriented 90° to the detector.

## Touchscreen


The NanoDrop Ultra comes with a built-in, 10-inch, high-resolution touchscreen preloaded with easy-to-use instrument control software. The touchscreen can tilt forward or back for optimal viewing.

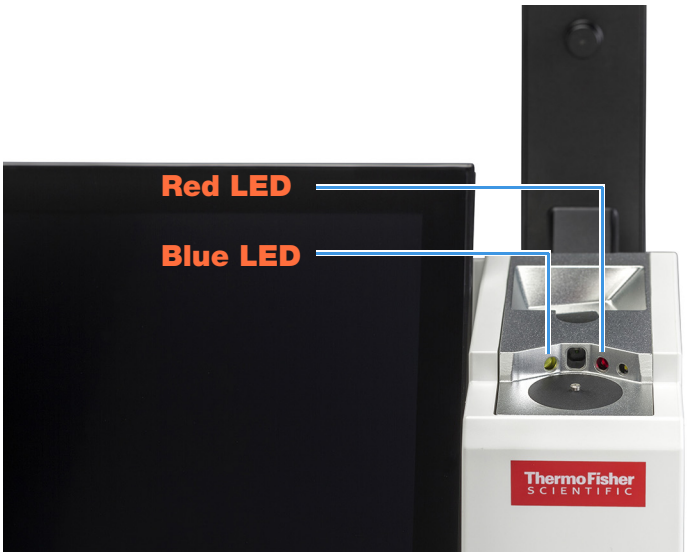
# Cuvette Holder


The NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL include a cuvette holder for measuring dilute samples, colorimetric assays, cell cultures and kinetic studies. The cuvette system has these features:

- extended lower detection limits
- 37 °C heater option for temperature-sensitive samples and analyses
- micro-stirring option to ensure sample homogeneity and support kinetic studies

For details, see Measure a Sample using a Cuvette.

## Fluorescence LEDs




The NanoDrop Ultra FL and NanoDrop Ultra$^{C}$ FL are enhanced with the inclusion of one red LED and one blue LED for fluorescence measurements. This addition allows users to perform fluorescence analysis, providing an extra dimension to the spectrophotometric capabilities of the instrument. The red and blue LEDs serve as excitation sources, enabling the detection of fluorescent emissions from samples that respond to these specific wavelengths. The fluorescence system has these features:

- **High Sensitivity**—more sensitive than absorbance–based quantification with the ability to detect down to 0.1 ng/µL dsDNA
- **Sample Prep Calculator**—integrated reagent calculator determines the amount of dye and buffer needed for the selected NanoDrop Ultra Fluorescence Assay
- **NanoDrop Ultra Fluorescence Assay Kits**—fluorescence assays to be used with built-in software applications for dsDNA and RNA detection

## USB-A port

One USB-A port located on the instrument rear panel.

## USB-B port

One USB-B port located on instrument rear panel.

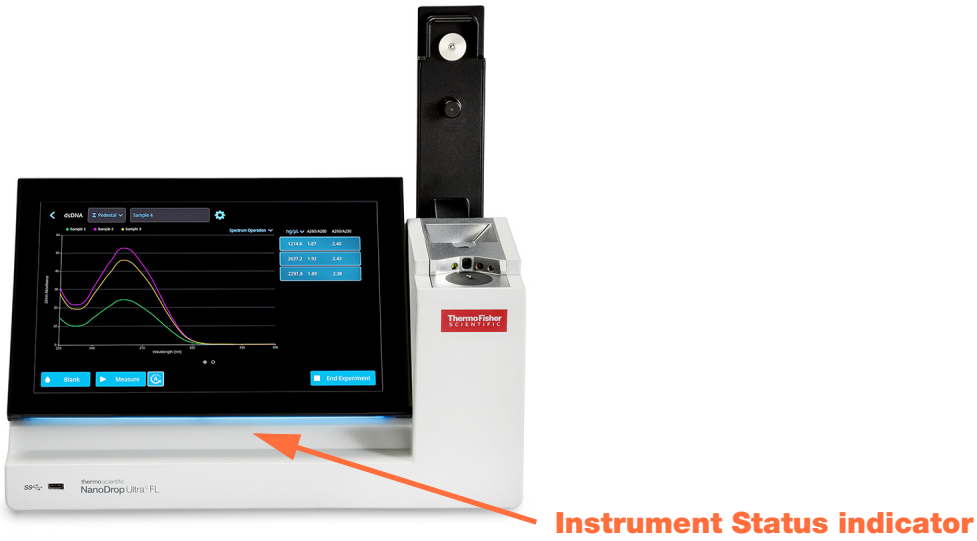
## USB-C ports

One USB-C port located on the front of the instrument and one USB-C port dedicated for power, located on the instrument rear panel.

## Wi-Fi™ and Bluetooth® Dongle port

One recessed USB-A port located on the instrument rear panel dedicated for Wi-Fi and Bluetooth connectivity. This port also acts as a standard USB-A port in the event that the Wi-Fi and Bluetooth dongle is not used.

## Instrument Status Indicator




The instrument status indicator located below the touchscreen will display specific colors and patterns to indicate current status of the instrument.

| Indicator | Instrument Status |
|---|---|
| Off | Powered off/Energy Saver Mode |
| Breathing white light | Powering on or initializing |
| Constant white light | Ready/standby |
| Sweeping blue light | Measuring |
| Constant blue light | Diagnostic - pass result<br>Measurement does not trigger Acclaro alert or Acclaro contaminant |
| Constant amber light | Diagnostic - conditional pass result<br>Measurement triggers Acclaro alert or Acclaro contaminant |
| Blinking amber light | Diagnostic - fail result |

# Accessories

This section lists the accessories for use with the NanoDrop Ultra instruments.

**Note** Some accessories listed below are not included with the purchase of NanoDrop Ultra spectrophotometers and fluorometers.

## NanoDrop Ultra USB Wi-Fi and Bluetooth Dongle

A USB Wi-Fi and Bluetooth dongle used to enable Wi-Fi and Bluetooth connectivity.


The NanoDrop Ultra USB W-Fi and Bluetooth Dongle is intended to be plugged into the recessed USB-A port in the back of the instrument.

## DYMO™ LabelWriter™ 550 USB Label Printer


Prints two 5/16-in x 4-in self-adhesive labels for transferring sample data directly into laboratory notebooks or posting on bulletin boards. The software allows printing of data from each sample measurement or from a group of samples logged and measured together.

The printer connects to the instrument (back panel) via a USB cable (included).

## PR-1 Pedestal Reconditioning Kit


Specially formulated conditioning compound that can be applied to the pedestals to restore them to a hydrophobic state (required to achieve adequate surface tension for accurate sample measurements). The kit includes conditioning compound and applicators. For more information, see Reconditioning the Pedestals.

## PV-1 Performance Verification Solution

Liquid photometric standard used to check instrument absorbance performance. For more information, see Performance Verification.

## FL-1 Fluorescence Verification Kit

Reagent based assay used to check instrument fluorescence performance. For more information, see Fluorescence Performance Check.

## NanoDrop Ultra dsDNA BR Fluorescence Assay

An assay kit that provides an accurate and selective method for the quantitation of sensitive double-stranded DNA samples. To be used with the dsDNA Fluorescence application.

## NanoDrop Ultra dsDNA HS Fluorescence Assay

An assay kit that provides an accurate and selective method for the quantitation of sensitive double-stranded DNA samples. To be used with the dsDNA Fluorescence application.

## NanoDrop Ultra RNA HS Fluorescence Assay

An assay kit that provides an accurate and selective method for the quantitation of sensitive RNA samples. To be used with the RNA Fluorescence application.

## NanoDrop Ultra Instrument Dust Cover

Transparent plastic cover protects instrument against dust and any liquid exposure while not in use.

## NanoDrop Ultra Microfiber Screen Wipe


Specialized cleaning cloth designed for safely and effectively cleaning the NanoDrop Ultra touch screen.

## USB-C Battery Pack

The NanoDrop Ultra instruments are designed to be compatible with USB-C portable chargers (75 Wh provides approximately 8 hours of run time).

# Instrument Detection Limits

## Absorbance-Based Detection Limits

| Measurement Location | Pathlength (mm) | Upper Detection Limit (10 mm Equivalent Absorbance) |
|---|---|---|
| Pedestal | 1.0 | 12.5 |
| | 0.2 | 62.5 |
| | 0.1 | 150 |
| | 0.05 | 300 |
| | 0.03 | 550 |
| Cuvette | 10 | 1.5 |
| | 5 | 3 |
| | 2 | 7.5 |
| | 1 | 15 |

## Fluorescence-Based Detection Limits

The fluorescence-based detection limits are dependent on the fluorescence assay used with the instrument. See the kit's protocol for more information.

The NanoDrop Ultra fluorescence kits have been optimized for microvolume measurements. Performance of other fluorescence dyes/reagents on the microvolume pedestal cannot be guaranteed.

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# Instrument Set up

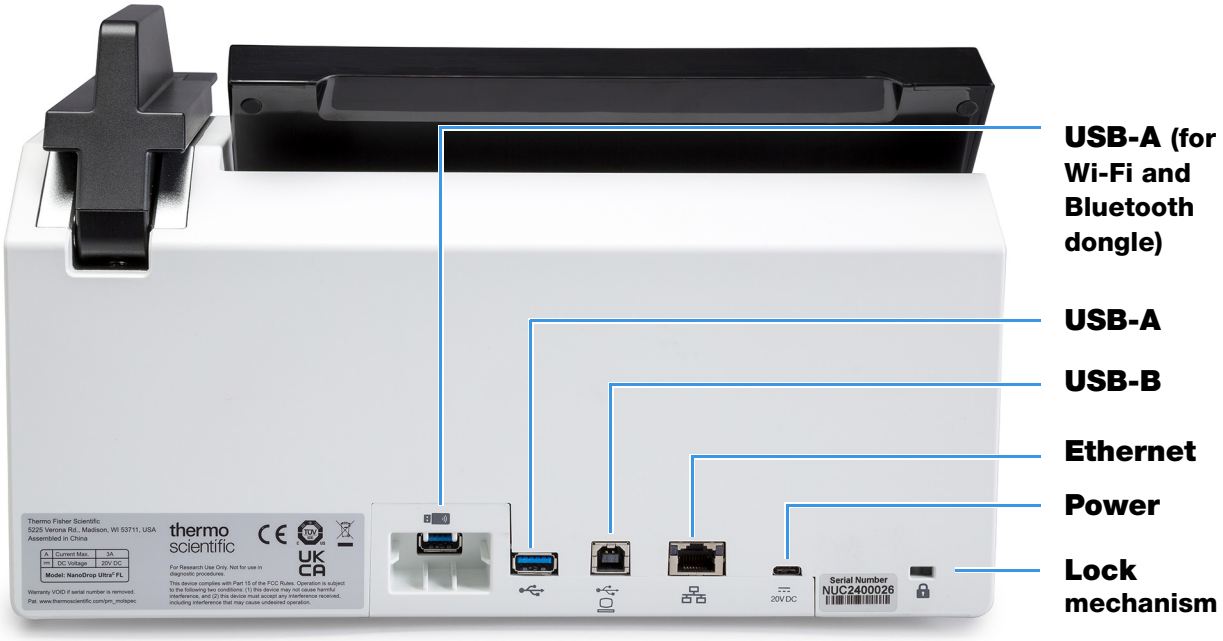
## Register Your Instrument

Register your instrument to receive e-mail updates on software and accessories for the NanoDrop Ultra instrument. An Internet connection is required for registration.

**To register your instrument**

From any PC that is connected to the Internet, use any web browser to navigate to www.thermofisher.com/nanodropsw. On the website, locate the “Register your instrument” button, and follow the instructions to register the instrument.

## Set Up Your Instrument



## Connect Power

**CAUTION** Avoid shock hazard. Each wall outlet used must be equipped with a ground. The ground must be a noncurrent-carrying wire connected to earth ground at the main distribution box.

Connect the provided power cord to a grounded wall outlet and then to the designated USB-C port on the back of the instrument. See "Power Cords" on page 388 for more information.

## Connect an Accessory

To connect a compatible printer or other compatible accessory such as a USB keyboard and/or mouse to the instrument, use any USB port on the instrument (front or back). See Accessories for information about accessories compatible with the NanoDrop Ultra instruments.

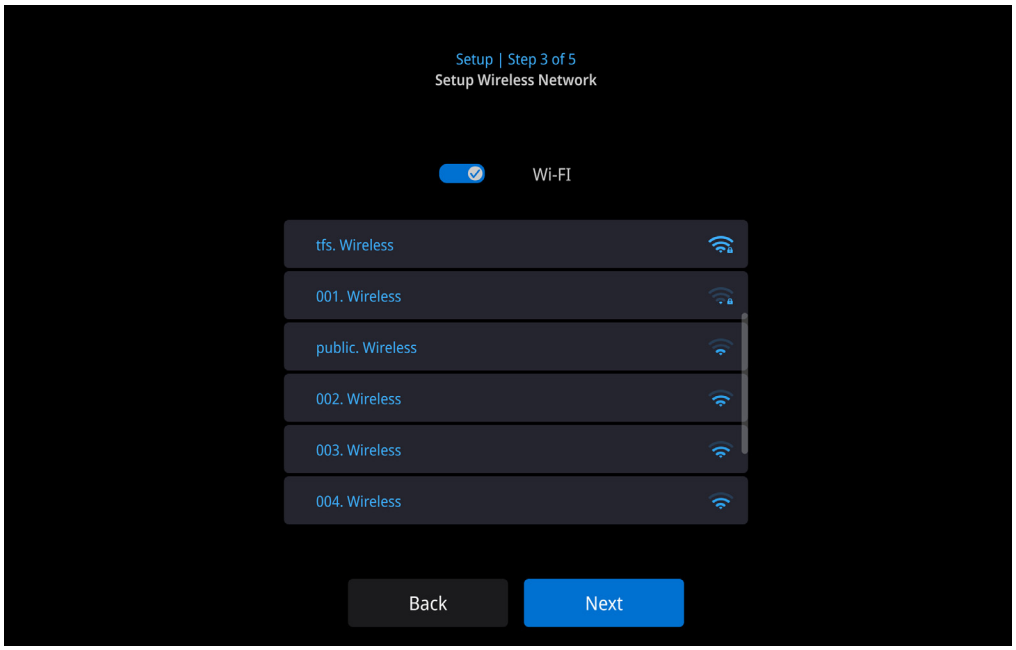
## Connect the USB Wi-Fi and Bluetooth Dongle


To connect the provided USB Wi-Fi and Bluetooth Dongle, use the dedicated recessed USB port on the back panel of the instrument. The Wi-Fi and Bluetooth features of this instrument will only function when a USB Wi-Fi and Bluetooth Dongle is connected.

## Out-of-Box Experience

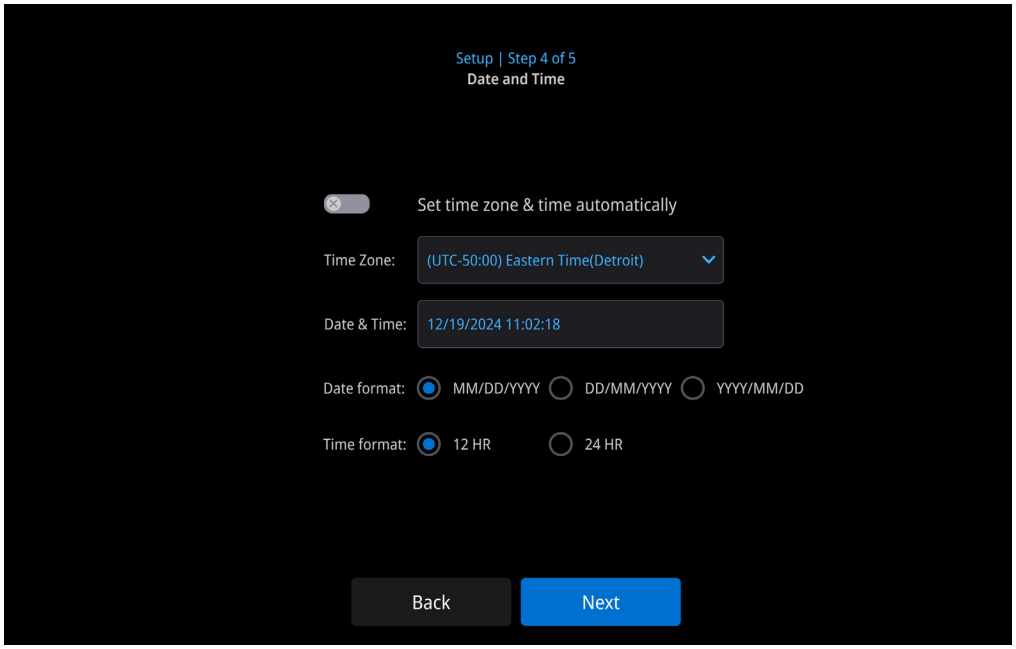
The Out-of-Box Experience (OOBE) is the initial setup process that you encounter when you first start a NanoDrop Ultra instrument. It is designed to help you configure essential settings, personalize your environment, and get your device ready for use.

1. Power on the instrument for the first time.
2. Read and **Accept** the End User License Agreement (EULA).
3. Select the desired language for the software from the provided drop-down menu and select **Next**.
4. If it is desired to setup a Wi-Fi connection at this time, select the toggle button next to Wi-Fi to move it to the right, it will turn blue and a checkmark will be displayed.
5. Select from the list of available Wi-Fi networks (input password if required, then select **OK**) then select **Next**.




See Instrument Settings in the Learning Center chapter for more information on how to connect to a Wi-Fi network.

7. If it is not desired to connect to a Wi-Fi network at this time, select **Next** without enabling Wi-Fi.
8. Select the desired date and time settings from the appropriate menus then select **Next**.




See Instrument Settings in the Learning Center chapter for more information on how to configure the date and time settings.

10. Select **Finish** to complete the OOBE setup. the instrument must restart for the changes to take effect.

## Set Up Bluetooth Connections

Use Bluetooth to connect the instrument to one or more Bluetooth (wireless) input devices such as a Bluetooth keyboard, mouse or barcode scanner.

**Note** Make sure the device is labeled “Bluetooth” and not just “wireless.” All Bluetooth devices are wireless but not all wireless devices will run with Bluetooth.

Ensure the NanoDrop Ultra Wi-Fi and Bluetooth Dongle is connected to the instrument before proceeding with setup. See “Connect the USB Wi-Fi and Bluetooth Dongle” on page 22.

See the section on bluetooth connectivity for additional details on connecting to a Bluetooth device

## Set up Wireless Connection

Ensure the NanoDrop Ultra USB Wi-Fi and Bluetooth Dongle is connected to the instrument before proceeding with setup. See “Connect the USB Wi-Fi and Bluetooth Dongle” on page 22.

See the section on Wi-Fi connectivity additional details on connecting to a Wi-Fi network.

## Set Up Cloud Connection

If the instrument is connected to a Cloud account, you can export data files to the following Cloud services: Thermo Fisher Connect, Google Drive, and Microsoft OneDrive. Multiple Cloud accounts can be added to the instrument allowing the operator the ability to select from the available list when exporting data.

**Note** An ethernet or Wi-Fi connection to a network with internet access is required for this feature.

See the section on cloud connectivity for additional details on connecting to a Cloud platform.

## Set Up Ethernet Connection

The instrument Ethernet port can be used to set up a wired connection between the instrument and an active network wall jack.

If the instrument is connected to a network wall jack, you can export data files to a network location, for example, in order to transfer them to another computer. You can define multiple network paths that the operator can select from when exporting data. See Export Settings for details. If the connected network has internet access, the ability to setup Cloud exporting and use online software updates are enabled.

### Parts needed:

- Shielded (straight through) Ethernet cable (CAT5e or newer is recommended)

### Set up Ethernet connection

1. Connect one end of Ethernet cable to the Ethernet port on the instrument back panel.


2. Connect the other end of the Ethernet cable to an active network wall jack.
3. When connected to a network via an Ethernet connection, the Ethernet Connectivity icon will appear at the top of the screen.

## Set Up USB Connection

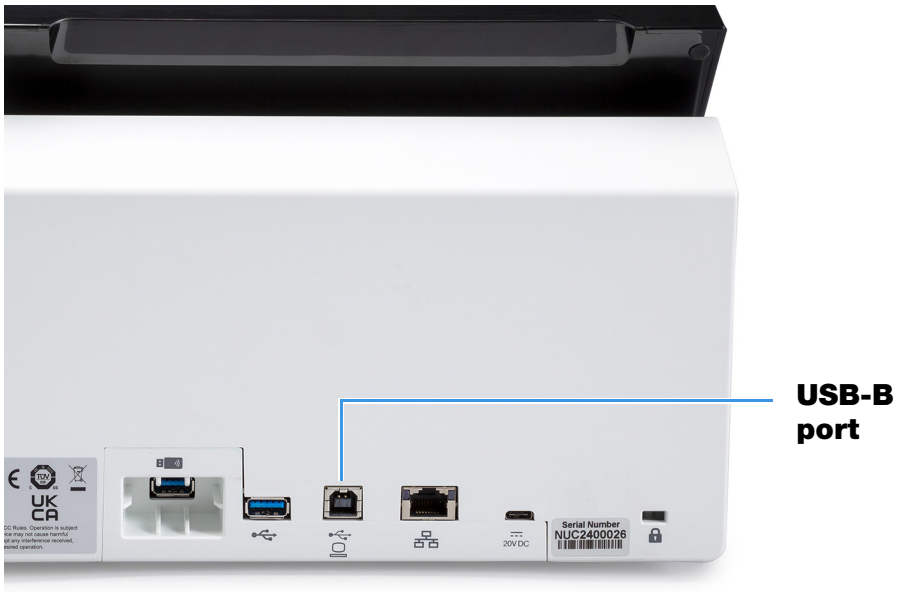
The instrument USB-B port can be used to set up a wired connection between the instrument and a personal computer (PC) or to connect to a USB printer.

### Parts needed:

- Standard USB-A to USB-B cable (not supplied)

### Set up USB connection

1. Connect the USB-B end of USB cable to the USB-B port on the instrument back panel.




2. Connect the USB-A end of the USB cable to either the computer or printer USB-A port.
3. When connected to a printer via a USB connection, a printer icon will appear at the top of the screen.

## Assess Instrument Connectivity

Use the System Status icon at the top right of the instrument home screen to quickly assess the instrument's connectivity status including Bluetooth and Wi-Fi. "System Status" on page 296 for more details.

# Operating Specifications

The instrument operates reliably when the room environment meets these specifications:

- operating temperatures: 5 °C - 35 °C (41 °F - 95 °F)
- relative humidity (non-condensing): 20-80%

Locate the instrument away from air vents and exhaust fans to minimize evaporation.

**Note** If operating the instrument at the low end of the recommended humidity range, use adequate sample volume to avoid evaporation.

# Computer Requirements

When controlling the instrument through a connected computer as opposed to the local touch screen, use the information below to select a compatible computer to operate the software.

### Required Windows Operating System

- Windows 10/11 Enterprise or Professional, build 1607 or greater

### Minimum hardware configuration

- 2.0 GHz dual-core processor enabled
- 4 GB RAM with system managed memory enabled
- 5 GB available on drive C
- Display resolution 1366 x 768

### Recommended hardware configuration

- 2.33 GHz 4-core processor enabled (or greater)
- 8 GB RAM with system managed memory enabled (or greater)
- 200 GB available on drive C (or greater)

# Update Software

Quickly and easily download and install the latest NanoDrop Ultra software and release notes from our website. Follow the steps to update or upgrade the software on your local instrument and/or install or update the NanoDrop Ultra software on a personal computer (PC). A PC with an Internet connection is required to download software.

See “Update Software” on page 350 for information on updating the NanoDrop Ultra software.

# Technical Support

## For U.S./Canada Support, please contact:

Thermo Fisher Scientific
3411 Silverside Road
Tatnall Building, Suite 100
Wilmington, DE 19810 U.S.A.

Telephone: 302 479 7707
Toll Free: 1 877 724 7690 (U.S. & Canada only)
Fax: 302 792 7155
E-mail: nanodrop@thermofisher.com
Website: www.thermofisher.com/nanodrop

## For International Support, please contact:

Contact your local distributor. For contact information go to:

http://www.thermofisher.com/NanoDropDistributors

If you are experiencing an issue with your system, refer to the troubleshooting information. If the issue persists, contact us. If you are outside the U.S.A. and Canada, please contact your local distributor.

If your instrument requires maintenance or repair, contact us or your local distributor.

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# Application Measurement Ranges

## Detection Limits for All Applications

**Note** Absorbance-based detection limits provided in the tables below are approximate and apply to micro-volume measurements only; they are based on the instrument's photometric absorbance range (10 mm equivalent) of 0–550 A. For measurements with 10 mm pathlength cuvettes, the photometric absorbance range is 0–1.5 A.

### Detection limits for absorbance-based applications

| Sample Type | Lower Detection Limit | Upper Detection Limit | Typical Reproducibility[a] |
|---|---|---|---|
| dsDNA (pedestal) | 1.0 ng/µL (pedestal)<br>0.20 ng/µL (cuvette) | 27,500 ng/µL (pedestal)<br>75 ng/µL (cuvette) | ±1.0 ng/µL for sample concentrations between 1.0 and 100 ng/µL samples;<br>±2% for samples >100 ng/µL |
| ssDNA | 0.66 ng/µL (pedestal)<br>0.13 ng/µL (cuvette) | 18,150 ng/µL (pedestal)<br>49.5 ng/µL (cuvette) | ±0.66 ng/µL for sample concentrations between 0.66 and 100 ng/µL samples;<br>±2% for samples >100 ng/µL |

| Sample Type | Lower Detection Limit | Upper Detection Limit | Typical Reproducibility[a] |
|---|---|---|---|
| RNA (pedestal) | 0.8 ng/µL (pedestal)<br>0.16 ng/µL (cuvette) | 22,000 ng/µL (pedestal)<br>60 ng/µL (cuvette) | ±0.8 ng/µL for sample concentrations between 0.8 and 100 ng/µL samples;<br>±2% for samples >100 ng/µL |
| DNA Microarray (ssDNA) | 0.66 ng/µL (pedestal)<br>0.13 ng/µL (cuvette) | 495 ng/µL (pedestal)<br>49.5 ng/µL (cuvette) | ±0.66 ng/µL for sample concentrations between 0.66 and 100 ng/µL samples;<br>±2% for samples >100 ng/µL |
| Purified BSA by Protein A280<br>IgG by Protein A280 | 0.03 mg/mL (pedestal)<br>0.006 mg/mL (cuvette)<br>0.02 mg/mL (pedestal)<br>0.003 mg/mL (cuvette) | 820 mg/mL (pedestal)<br>402 mg/mL (pedestal) | ±0.03 mg/mL (for 0.03–10 mg/mL samples);<br>±2% for samples >10 mg/mL |
| Purified BSA by Proteins & Labels | 0.06 mg/mL (pedestal)<br>0.006 mg/mL (cuvette) | 19 mg/mL (pedestal) | ±0.06 mg/mL for 0.06–10 mg/mL samples |
| Protein BCA | 0.125 mg/mL (20:1 reagent/sample volume)<br>0.02 mg/mL (1:1 reagent/sample volume) | 2.0 mg/mL<br>0.20 mg/mL | 2% over entire range<br>0.01 mg/mL over entire range |
| Protein Lowry | 0.2 mg/mL | 1.5 mg/mL | 2% over entire range |
| Protein Bradford | 100 µg/mL (30:1 reagent/sample volume)<br>15 µg/mL (1:1 reagent/sample volume) | 1000 µg/mL<br>100 µg/mL | ±25 µg/mL for 100–500 µg/mL samples<br>±5% for 500–1000 µg/mL samples<br>±4 µg/mL for 15–50 µg/mL samples<br>±5% for 50–100 µg/mL samples |
| Protein Pierce 660 | 50 µg/mL (15:1 reagent/sample volume)<br>25 µg/mL (7.5:1 reagent/sample volume) | 2000 µg/mL<br>1000 µg/mL | ±3 µg/mL for 50–125 µg/mL samples<br>±2% for samples > 125 µg/mL<br>±3 µg/mL for 25–125 µg/mL samples<br>±2% for samples >125 µg/mL |

[a] Based on five replicates (SD=ng/µL; CV=%)

**Note** To minimize instrument error with highly concentrated samples, make dilutions to ensure that measurements are made within these absorbance limits:

- For micro-volume measurements, maximum absorbance at 260 nm (for nucleic acids) or 280 nm (for proteins) should be less than 62.5 A.
- For measurements with 10 mm pathlength cuvettes, maximum absorbance at 260 nm (or 280 nm for proteins) should be less than 1.5 A, which is approximately 75 ng/µL dsDNA.

## Detection limits (absorbance) for pre-defined fluorescent dyes

| Sample Type | Lower Detection Limit | Upper Detection Limit[a] | Typical Reproducibility[b] |
|---|---|---|---|
| Cy3, Cy3.5, Alexa Fluor 555, Alexa Fluor 660 | 0.2 pmol/µL (pedestal) | 100 pmol/µL (pedestal) | ±0.20 pmol/µL for sample concentrations between 0.20 and 4.0 pmol/µL; ±2% for samples >4.0 pmol/µL |
| Cy5, Cy5.5, Alexa Fluor 647 | 0.12 pmol/µL (pedestal) | 60 pmol/µL (pedestal) | ±0.12 pmol/µL for sample concentrations between 0.12 and 2.4 pmol/µL; ±2% for samples >2.4 pmol/µL |
| Alexa Fluor 488, Alexa Fluor 594 | 0.4 pmol/µL (pedestal) | 215 pmol/µL (pedestal) | ±0.40 pmol/µL for sample concentrations between 0.40 and 8.0 pmol/µL; ±2% for samples >8.0 pmol/µL |
| Alexa Fluor 546 | 0.3 pmol/µL (pedestal) | 145 pmol/µL (pedestal) | ±0.30 pmol/µL for sample concentrations between 0.30 and 6.0 pmol/µL; ±2% for samples >6.0 pmol/µL |

[a] Values are approximate

[b] Based on five replicates (SD=ng/µL; CV=%)

# Detection Limits for Fluorescence-based Applications

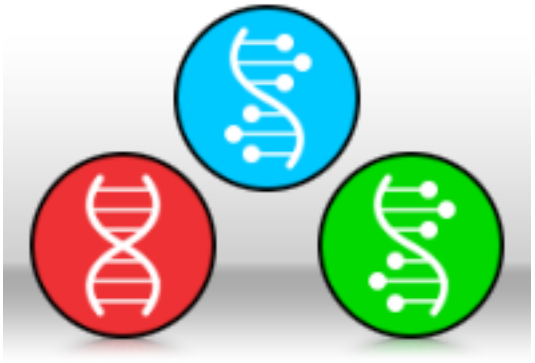
The fluorescence-based detection limits are dependent on the fluorescence assay used with the instrument. See the kit's protocol for more information.

**Note** The NanoDrop Ultra Fluorescence Assays have been optimized for microvolume measurements. Performance of other fluorescence dyes / reagents on the microvolume pedestal cannot be guaranteed.

# Nucleic Acid Applications

## dsDNA, ssDNA or RNA

Measures the concentration of purified dsDNA, ssDNA or RNA samples that absorb at 260 nm.



Measure dsDNA, ssDNA or RNA

Reported Results

Settings

Kit Editor

Detection Limits

Calculations

### Best practices for nucleic acid measurements

- Isolate and purify nucleic acid samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA, free nucleotides, proteins, some buffer components and dyes. See Preparing Samples for more information.

**Note** Extraction reagents such as guanidine, phenol, and EDTA contribute absorbance between 230 nm and 280 nm and will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.

- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 260 nm), you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - If possible, heat highly concentrated or large molecule samples, such as genomic or lambda DNA, to 63 °C (145 °F) and gently (but thoroughly) vortex before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

### Related Topics

- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Best Practices for Micro-Volume Measurements
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

## Measure dsDNA, ssDNA or RNA

Use the dsDNA, ssDNA and RNA applications to quantify purified double-stranded (ds) or single-stranded (ss) DNA or RNA samples. These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

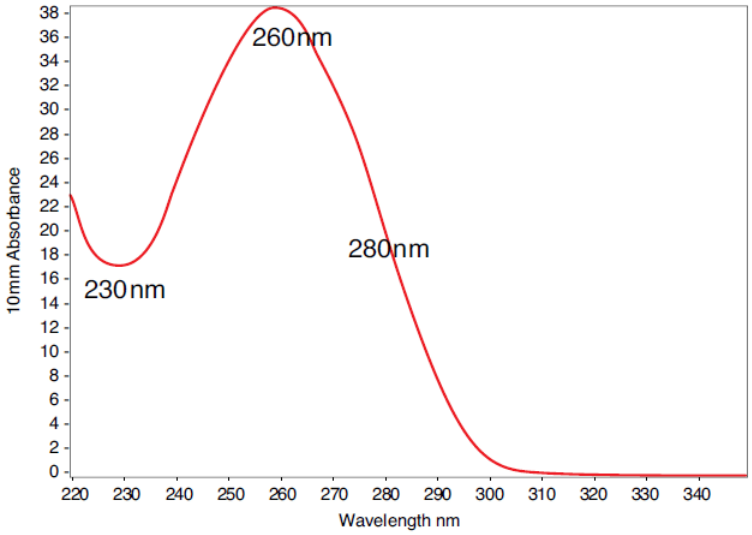
1. From the home screen, select the **Nucleic Acids** tab, then select **dsDNA, ssDNA or RNA**, depending on the samples to be measured.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
5. Select **Blank** and wait for the measurement to complete.

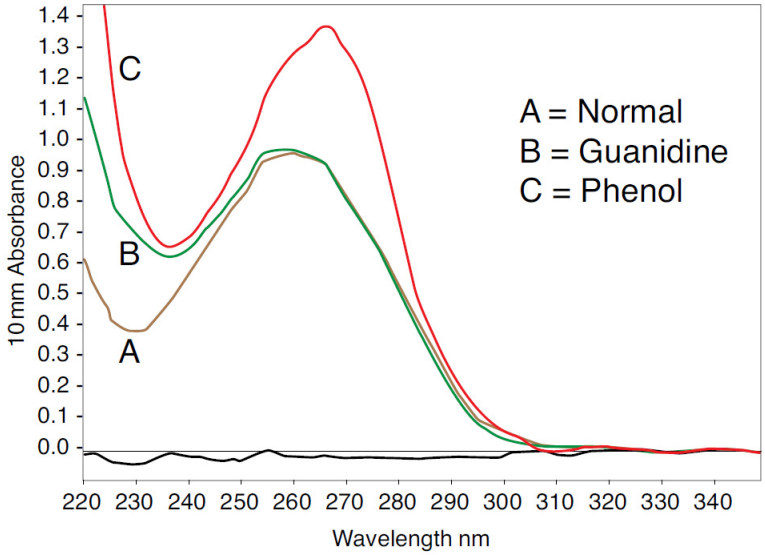
   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
7. Pipette 1-2 µL sample solution onto the pedestal, or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.
   - Cuvette: Select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**Typical nucleic acid spectrum**

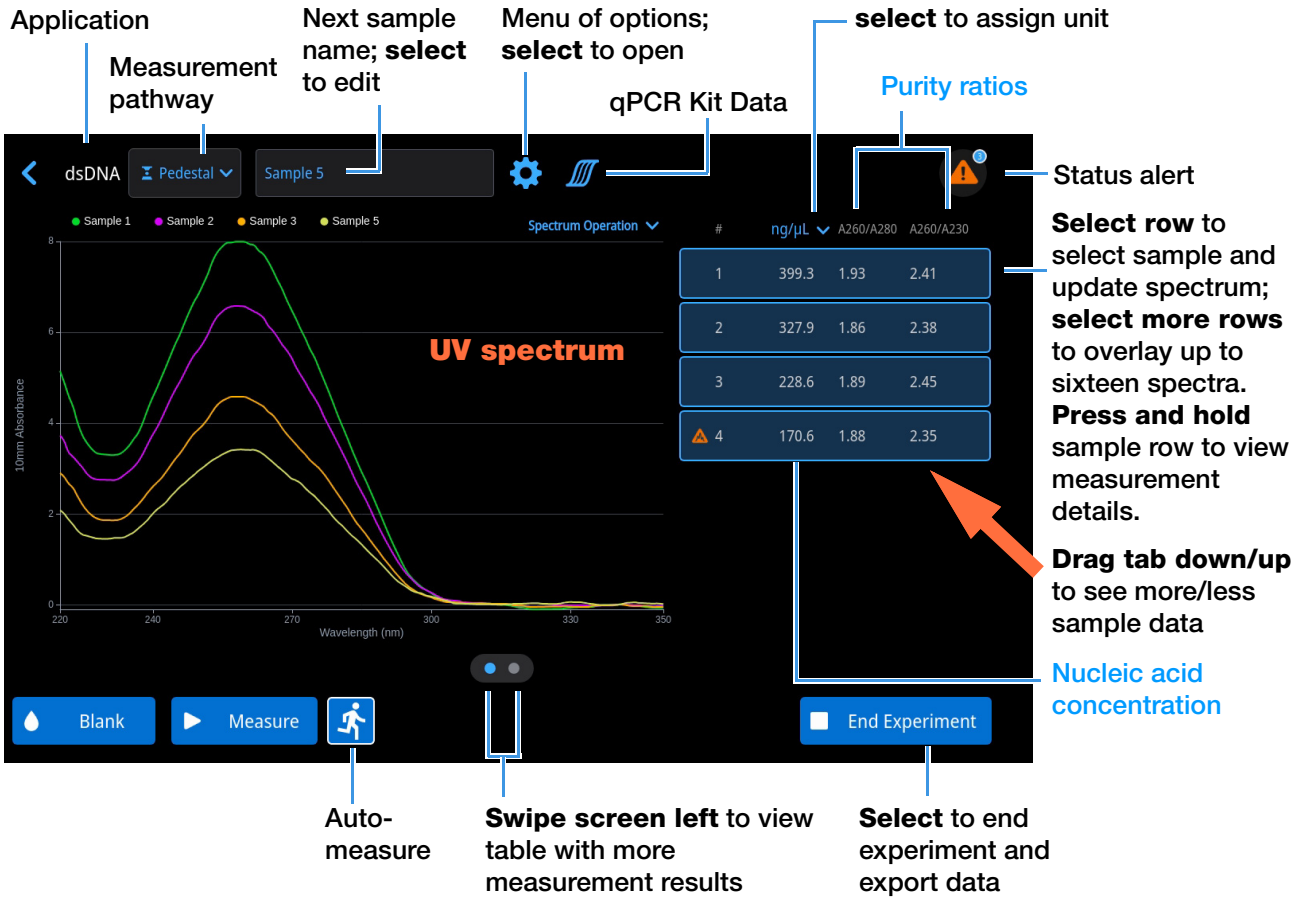



**Comparison of nucleic acid spectra with and without two common contaminants**

# Reported Results

## dsDNA measurement screen (local control)

For each measured sample, the dsDNA, ssDNA and RNA applications show the UV absorbance spectrum and a summary of the results. The following is an example of the measurement screen of the NanoDrop Ultra local control software:




**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## dsDNA measurement screen (PC control)

For each measured sample, the dsDNA, ssDNA and RNA applications show the UV absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## qPCR Kit Data

The NanoDrop Ultra software includes an integrated qPCR calculator designed to swiftly determine the precise amount of your sample required for downstream qPCR reactions. When selecting the qPCR Kit Data icon at the top of the measurement screen, a summary of the calculations will be displayed, these calculations are specific to the Kit that was selected during setup. The following is an example of the qPCR Kit Data screen on the NanoDrop Ultra local control software screen:


### Dilution Required

If it necessary to dilute the nucleic acid before adding it to the reaction mix, follow the recommended dilution scheme outlined in the **Dilution Required** section.

If the **Dilution Required** section is empty, no dilution is needed before adding the nucleic acid to the reaction mix.

### Kit Reaction

Follow recommended volumes in the **Kit Reaction** section to achieve the desired Final Template Concentration in the kit reaction.

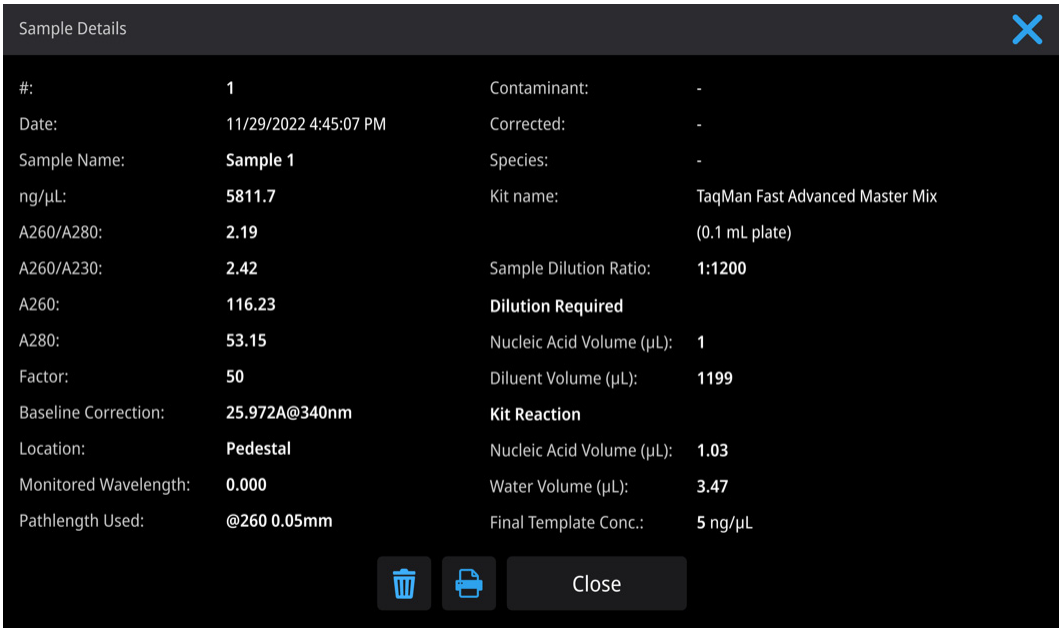
**Note** Final Template Concentration may be modified.

If a dilution is required in the Dilution Required section, the Nucleic Acid Volume called for in the Kit Reaction section will indicate the required amount of the diluted nucleic acid product to add to the reaction.

If a dilution is not required in the Dilution Required section, the Nucleic Acid Volume called for in the Kit Reaction section will indicate the required amount of the original nucleic acid sample to add to the reaction.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

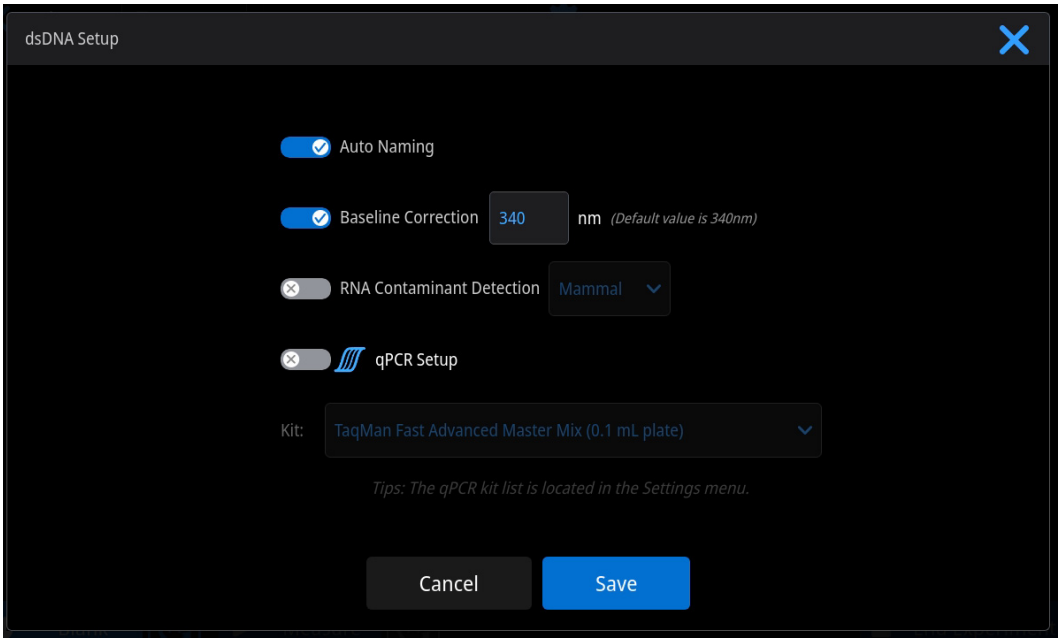



- date (date and time sample measurement was taken)
- sample name
- # (sample number)
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction
- location
- monitored wavelength
- pathlength used
- contaminant
- corrected nucleic acid concentration

- species
- kit name
- sample dilution ratio
- dilution required: nucleic acid volume (µL)
- dilution required: diluent volume (µL)
- kit reaction: nucleic acid volume (µL)
- kit reaction: water volume (µL)
- kit reaction: final template conc.
- stirrer
- heater
- measured temperature
- target temperature

## Settings

The dsDNA, ssDNA, or RNA Setup screen appears after you select the respective application from the Nucleic Acids tab on the home screen. To show the dsDNA, ssDNA or RNA settings, from the dsDNA, ssDNA or RNA measurement screen, select the respective Setup icon .



| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name "sample" followed by the number sample in the sequence. For example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |
| RNA Contaminant Detection | On or off<br><br>Use the drop-down menu to select between mammal, plant, or bacteria | When enabled, RNA Contaminant Detection/DNA Contaminant detection will apply mathematical models to predict the amount of RNA contaminant in dsDNA or dsDNA in RNA. These models are specific to the source of the nucleic acid. If you are measuring nucleic acid from a source for which we do not have a mathematical model, leave this feature off. |
| qPCR Setup | On or off<br><br>Use the drop-down menu to select one of the available qPCR kits. | When enabled, the software automatically calculates sample volumes needed for a qPCR reaction based on sample concentration and the qPCR kit selected at setup. |

## Detection Limits

The lower detection limits and reproducibility specifications for nucleic acids are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the user-defined extinction coefficients.

### To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

**Note** For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

### Related Topics

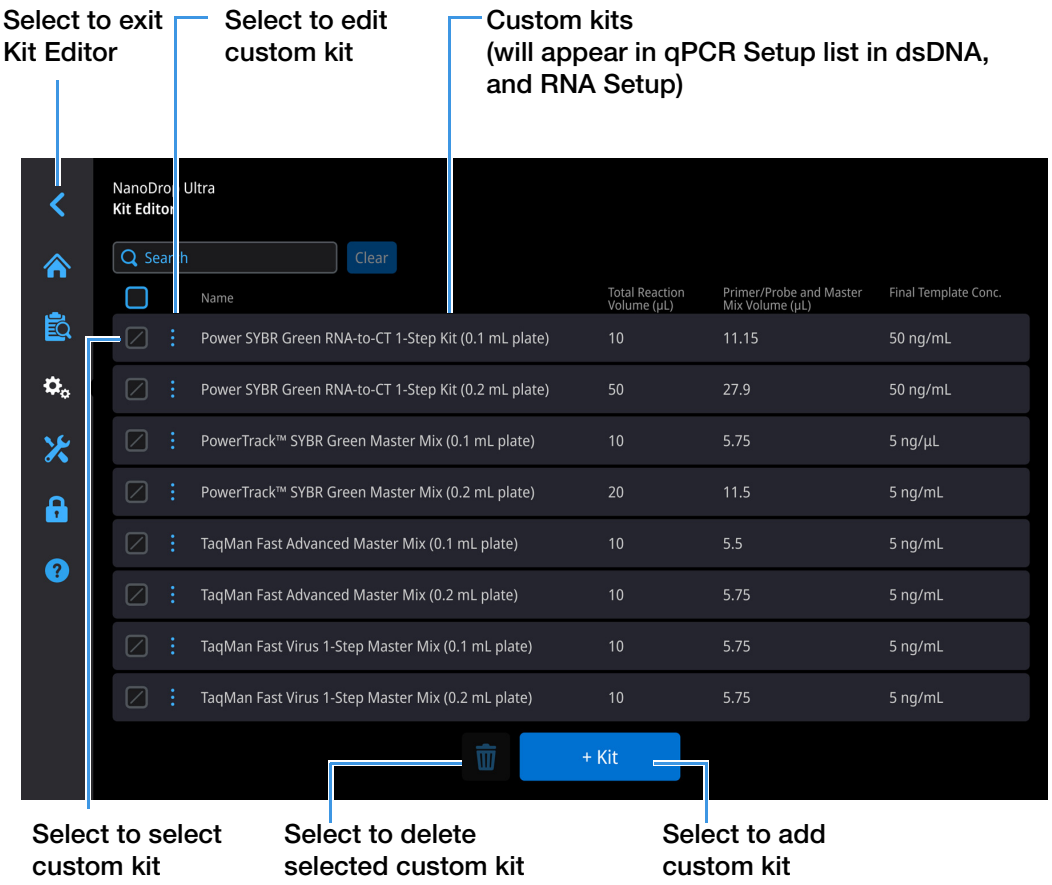
- Detection Limits for All Applications

## Kit editor

Use the **Kit Editor** to add a custom qPCR kit to the list of available kits in Settings for Nucleic Acid Measurements.

### To access the Kit Editor:

- From the software home screen, select **Settings** > **Kit Editor**.




Kit Editor as seen on the NanoDrop Ultra local control software

These operations are available from the Kit Editor:

### Add custom kit

1. In Kit Editor, select 

to show the **Add Kit** box.
2. Enter a unique name for the new kit (from local control, tap field to display keyboard, tap **Done** key to close keyboard).
3. Enter the Total Reaction Volume (µL), Primer/Probe and Master Mix Volume (µL), and Final Template Concentration with it's respective units for the kit in their respective fields.
4. Select **Save** to close the **Add Kit** window.

The new custom kit appears in the Kit list in dsDNA, and RNA Setup.

### Edit custom kit

1. In Kit Editor, select  in the row of the desired kit that needs to be edited.
2. Select 

to show the Edit Kit window.
3. Edit all available fields. For predefined kits, only the Final Template Concentration can be edited.
4. Select **Save**.

### Delete custom kit

1. In Kit Editor, select one or more custom kits to delete by using the check boxes to the left of each desired kit.
2. Select  .
3. Confirm deletion of the custom kit(s) by selecting **Yes**.

# Calculations

The Nucleic Acid applications use a modification of the Beer-Lambert equation (shown at right) to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

### Extinction Coefficients vs Factors

Using the terms in the Beer-Lambert equation, factor (f) is defined as:

$$\text{factor (f)} = 1/(\varepsilon * b)$$

where:
$\varepsilon$ = wavelength-dependent molar extinction coefficient in ng-cm/µL
b = **sample pathlength** in cm

As a result, analyte concentration (c) is calculated as:

$$c = A * [1/(\varepsilon * b)]$$

or

$$c = A * f$$

where:
c = analyte concentration in ng/µL
A = absorbance in absorbance units (A)
f = factor in ng-cm/µL (see below)

For the dsDNA, ssDNA and RNA applications, the generally accepted factors for nucleic acids are used in conjunction with Beer's Law to calculate sample concentration. For the Custom Factor application, the user-specified factor is used.

### Factors Used

- **dsDNA** (factor = 50 ng-cm/µL)
- **ssDNA** (factor = 33 ng-cm/µL)
- **RNA** (factor = 40 ng-cm/µL)
- **Custom Factor** (user entered factor between 15 ng-cm/µL and 150 ng-cm/µL

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

**Note**: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

### A260 absorbance

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

### A230 and A280 absorbance

- Normalized and baseline-corrected (if selected) absorbance values at 230 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized. (see Cuvette Settings)
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

- **Nucleic acid concentration**. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~1.8 is generally accepted as "pure" for DNA (~2.0 for RNA). Acidic solutions may under represent the reported value by 0.2-0.3; the opposite is true for basic solutions.
- **A260/A230 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm. An A260/A230 purity ratio between 1.8 and 2.2 is generally accepted as "pure" for DNA and RNA.

**Note**: Although purity ratios are important indicators of sample quality, the best quality indicator quality is functionality in the downstream application of interest (e.g., real-time PCR).

- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration
- **Contaminant** - If a contaminant was identified by the Acclaro software, the contaminant will be displayed in this column.
- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays whether the measurement was taken from the pedestal or cuvette mode.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.

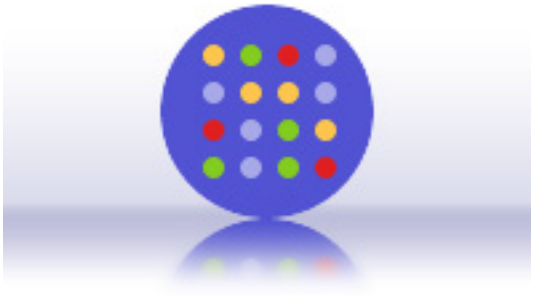
- **Contaminant**. If a contaminant was identified by the Acclaro software, the contaminant will be displayed in this column.
- **Corrected**. Displays the corrected analyte concentration determined using the Acclaro software, if one is available.
- **Species**. The species of nucleic acid contaminant detected by the Acclaro software
- **Kit name**. Provides the name of the qPCR kit used to determine qPCR recipe calculations, if one was selected during setup.
- **Sample Dilution Ratio**. When dilution of the sample is recommended prior to qPCR, displays the volume of the measured sample relative to the final volume of the dilution.
- **Dilution required: diluent volume (µL)**. When dilution of the sample is recommended prior to qPCR, displays the volume of the original nucleic acid sample to add to the dilution tube.
- **Dilution required: diluent volume (µL)**. When dilution of the sample is recommended prior to qPCR, displays the volume of diluent to add to the dilution tube.
- **Kit reaction: nucleic acid volume (µL)**. If no dilution is recommended in the Dilution Required fields, this indicates the volume of original nucleic acid sample to add to the qPCR reaction tube. If a dilution was recommended in the Dilution Required fields, this indicates the volume of the nucleic acid dilution tube to add to the qPCR reaction tube.
- **Kit reaction: water volume (µL)**. Displays the volume of water to add to the qPCR reaction tube.
- **Kit reaction: final template conc**. Displays the concentration of the nucleic acid sample after it's been added to the qPCR reaction tube.

- **Stirrer**. "Off" will be displayed when the stirrer function on a cuvette model is not used. When the stirrer function is used, the stir speed is displayed.
- **Heater**. "Off" or "On" will be displayed to show if the cuvette port was heated during the measurement.
- **Measured Temperature**. Temperature of the cuvette port during the measurement will be displayed.
- **Target Temperature**. Desired temperature of the cuvette port will be displayed.

# Microarray

Measures the concentration of purified nucleic acids that have been labeled with up to two fluorescent dyes for use in downstream microarray applications.





## Measure Microarray

Use the Microarray application to quantify nucleic acids that have been labeled with up to two fluorescent dyes. The application reports nucleic acid concentration, an A260/A280 ratio and the concentrations and measured absorbance values of the dye(s), allowing detection of dye concentrations as low as 0.2 picomole per microliter.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

### Procedure

1. From the home screen, select the **Nucleic Acids** tab, then select **Microarray**.

2. Configure any of the setup options if desired and select **Save**.

   **Tip**: Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.

3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.

   – When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.

   – When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.

4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.

5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.

7. Pipette 1-2 µL sample solution onto the pedestal, or insert the sample cuvette into the cuvette holder.

8. Start the sample measurement:

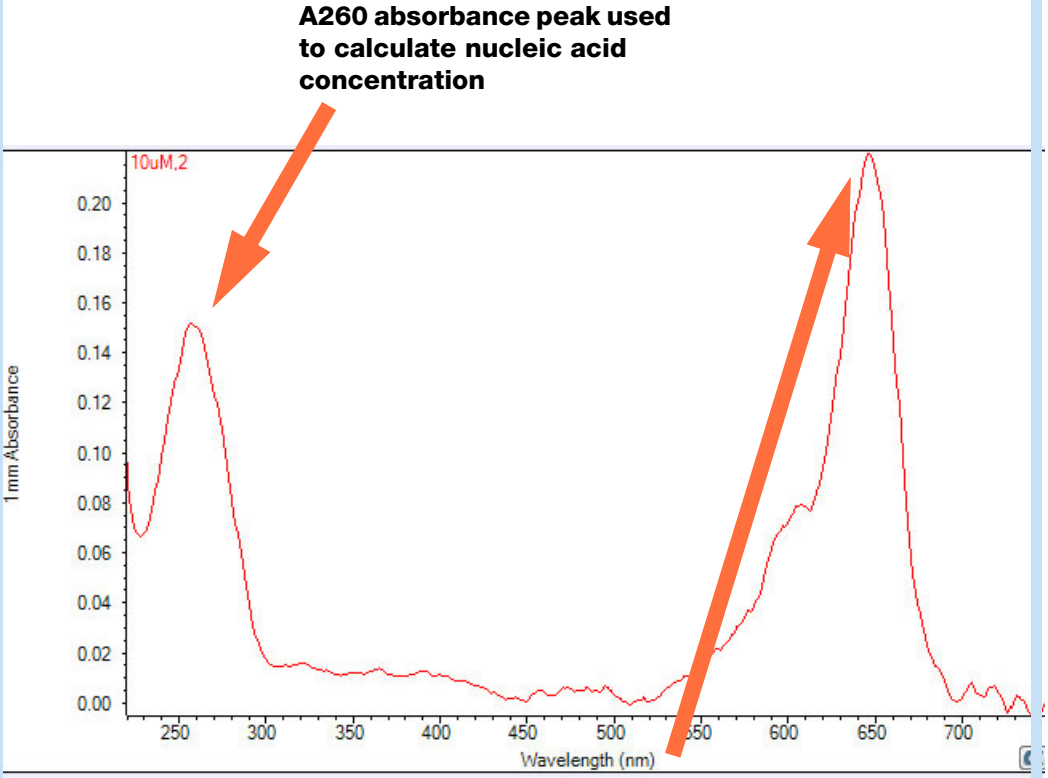
   – Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.

   – Cuvette: Select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.

10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**Typical microarray spectrum**

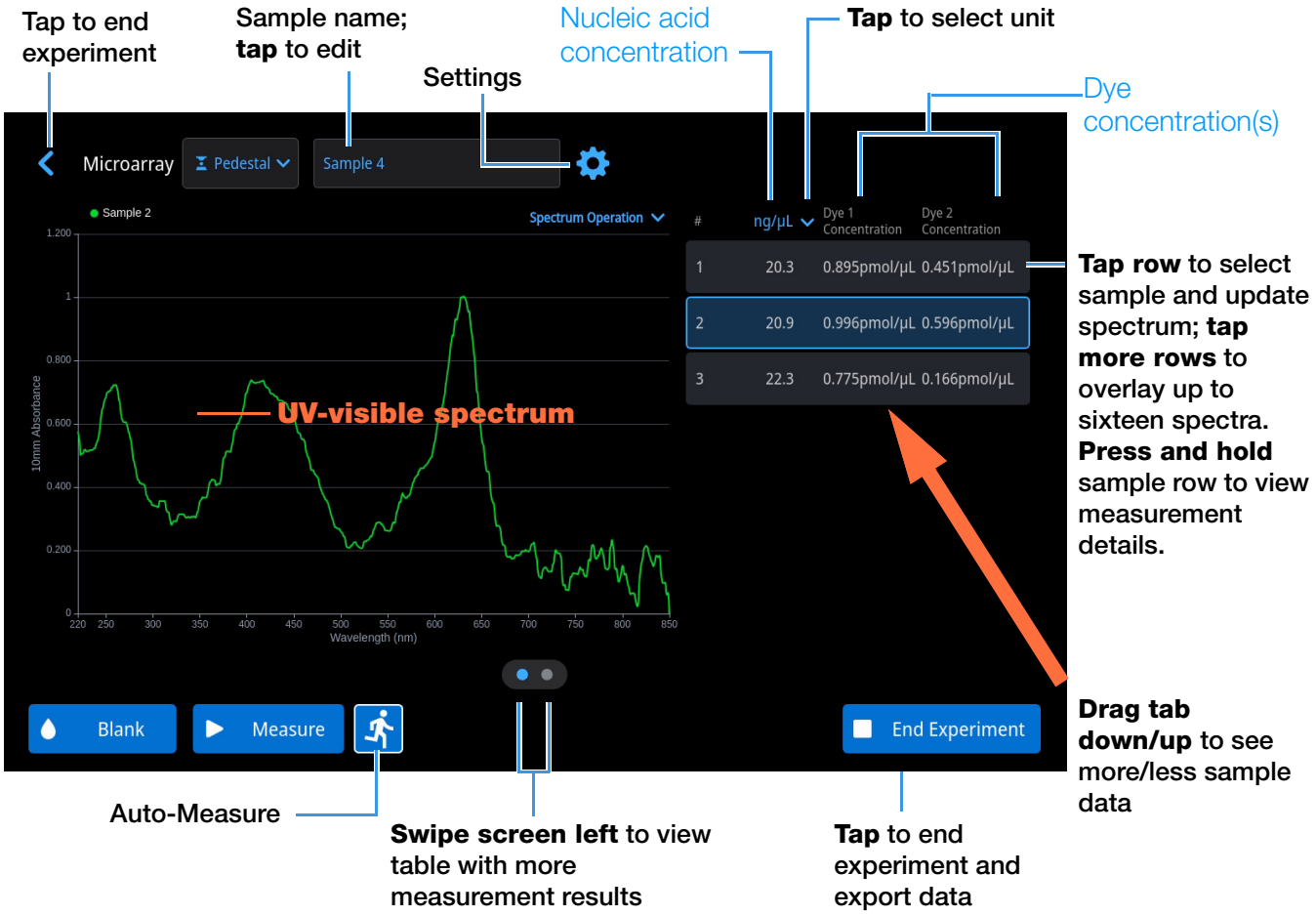
## Related Topics

- Best Practices for Nucleic Acid Measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Best Practices for Micro-Volume Measurements
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Microarray measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the measurement screen of the NanoDrop Ultra local control software:




**Note**

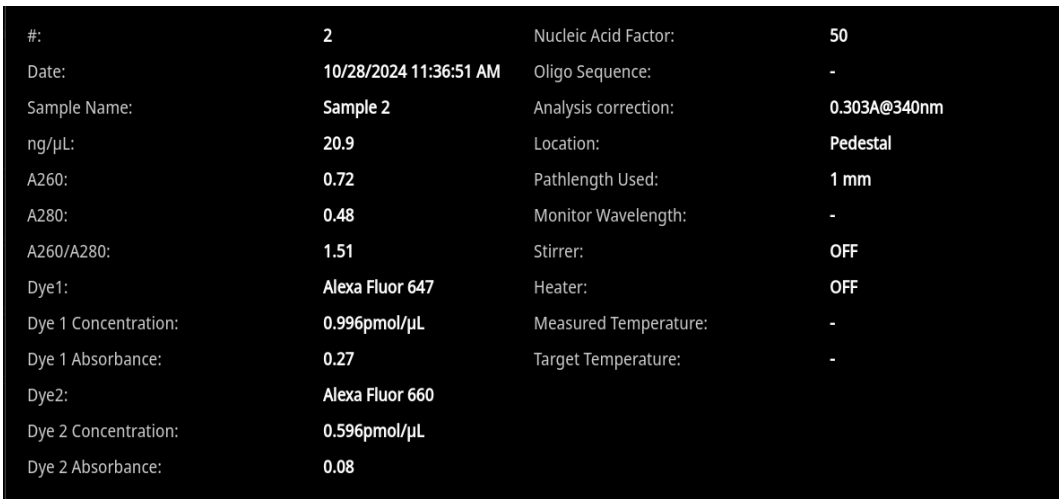
- A baseline correction is performed at 850 nm (absorbance value at 850 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Microarray measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



| | | | |
|---|---|---|---|
| #: | 2 | Nucleic Acid Factor: | 50 |
| Date: | 10/28/2024 11:36:51 AM | Oligo Sequence: | - |
| Sample Name: | Sample 2 | Analysis correction: | 0.303A@340nm |
| ng/µL: | 20.9 | Location: | Pedestal |
| A260: | 0.72 | Pathlength Used: | 1 mm |
| A280: | 0.48 | Monitor Wavelength: | - |
| A260/A280: | 1.51 | Stirrer: | OFF |
| Dye1: | Alexa Fluor 647 | Heater: | OFF |
| Dye 1 Concentration: | 0.996pmol/µL | Measured Temperature: | - |
| Dye 1 Absorbance: | 0.27 | Target Temperature: | - |
| Dye2: | Alexa Fluor 660 | | |
| Dye 2 Concentration: | 0.596pmol/µL | | |
| Dye 2 Absorbance: | 0.08 | | |

- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- A260
- A260/A280
- A280
- dye 1/dye 2 absorbance
- dye 1/dye 2 concentration
- dye 1/dye 2 name
- sample type
- analysis correction
- factor

- oligo sequence
- location
- monitor wavelength
- pathlength used
- stirrer
- heater
- measured temperature
- target temperature

## Settings

The Microarray Setup screen appears after you select the Microarray application from the Nucleic Acids tab on the home screen. To show the Microarry settings from the Microarray measurement screen, select Microarray Setup  .

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Sample type and Factor | dsDNA (with non-editable factor of 50 ng-cm/µL) | Widely accepted value for double-stranded DNA |
| | ssDNA (with non-editable factor of 33 ng-cm/µL) | Widely accepted value for single-stranded DNA |
| | RNA (with non-editable factor of 40 ng-cm/µL) | Widely accepted value for RNA |
| | Oligo DNA with non-editable calculated factor in ng-cm/µL | Factor calculated from user-defined DNA base sequence. When selected, available DNA base units (i.e., G, A, T, C) appear as keys. Define sequence by tapping appropriate keys. Factor is calculated automatically based on widely accepted value for each base unit. |

| Setting | Available Options | Description |
|---|---|---|
| | Oligo RNA with non-editable calculated factor in ng-cm/µL | Factor calculated from user-defined RNA base sequence. When selected, available RNA base units (i.e., G, A, U, C) appear as keys. Define sequence by tapping appropriate keys. Factor is calculated automatically based on widely accepted value for each base unit. |
| | Custom (with user-specified factor in ng-cm/µL) | Enter factor between 15 ng-cm/µL and 150 ng-cm/µL |
| Dye 1/Dye 2 Type[a] | Cy3, 5, 3.5, or 5.5, Alexa Fluor 488, 546, 555, 594, 647, or 660 | Select pre-defined dye(s) used to label sample material, or one that has been added using Dye Editor. |
| Dye 1/Dye 2 Unit | picomoles/microliter (pmol/uL), micromoles (uM), or millimoles (mM) | Select unit for reporting dye concentrations |
| Analysis Correction[b] | On or off<br><br>Enter analysis correction wavelength in nm or use default value (340 nm) | Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction. |

[a] To add a custom dye or edit the list of available dyes, use the Dye/Chromophore Editor.

[b] The Analysis Correction affects the calculation for nucleic acid concentration only.

## Dye/chromophore editor

Use the Dye/Chromophore Editor to add a custom dye to the list of available dyes in Microarray Setup or Proteins & Labels Setup. You can also specify which dyes are available in that list.

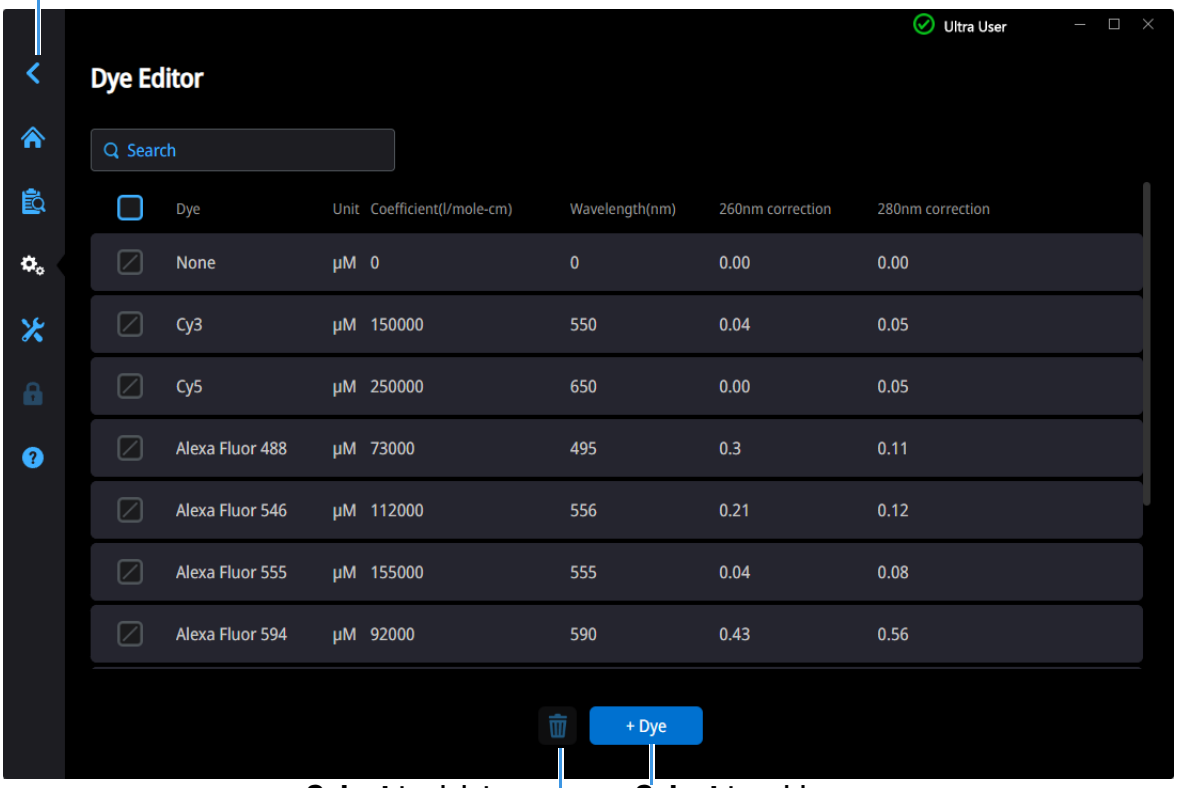
To access the Dye/Chromophore Editor:

- From the home screen, select **Settings**  , then select **Dye Editor**

**Dye Editor**
(PC control)

**Select** to exit Dye Editor

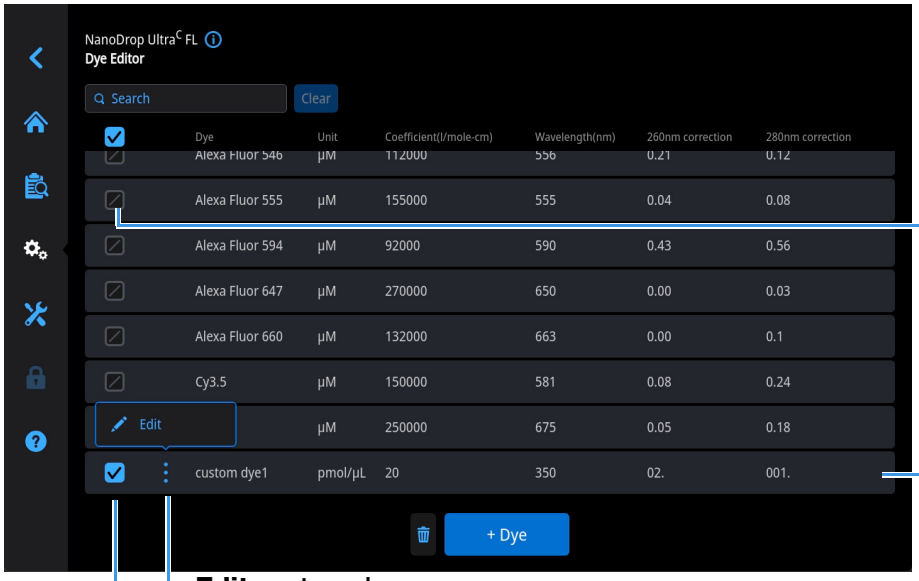
Selected dyes (will appear in Dye1 and Dye2 lists in Microarray Setup or Proteins & Labels Setup)




**Select** to delete selected custom dye

**Select** to add custom dye

**Dye Editor**
(local control)




Locked dye (pre-defined; cannot be edited or deleted)

Custom dye (user-defined; can be edited or deleted)

**Edit** custom dye

**Select** custom dye

These operations are available from the Dye/Chromophore Editor:

### Add custom dye

1. In the Dye Editor, select + Dye to show the Add Dye box.
2. Enter unique **Name** for new dye (tap field to display keyboard when using the local control software, select **Done** key to close keyboard).
3. Select default **Unit** that will be used to display dye concentration.
4. Enter dye's **Extinction Coefficient** (or molar absorptivity constant) in L/mole-cm (typically provided by dye manufacturer).
5. Specify **Wavelength** in nm (between 350 nm and 840 nm) that will be used to measure dye's absorbance.
6. Specify dye's correction values at 260 nm and 280 nm
7. Select **Save** to close the Add Dye box.

   The new custom dye appears in the **Dye 1** and **Dye 2** lists in Microarray Setup and Proteins and Labels Setup.

**Note** To determine dye correction values (if not available from dye manufacturer):

- use instrument to measure pure dye and note absorbance at 260 nm, 280 nm and at analysis wavelength for dye (see above)
- calculate ratio of $A_{260}/A_{\text{dye wavelength}}$ and enter that value for 260 nm Correction
- calculate ratio of $A_{280}/A_{\text{dye wavelength}}$ and enter that value for 280 nm Correction

When a custom dye is selected before a measurement, the dye's absorbance and concentration values are reported and the corrections are applied to the measured sample absorbance values, and to the resulting sample concentrations and purity ratios.

### Edit custom dye

**Tip** Dyes pre-defined in the software cannot be edited.

1. In Dye Editor, select ⋮ in the row of the desired custom dye that needs to be edited.

2. Select 

.
3. Edit any entries or settings.
4. Select **Save**.

### Delete custom dye

**Tip** Dyes pre-defined in the software cannot be deleted.

1. In Dye Editor, select one or more custom dyes to delete by using the check boxes to the left of each desired custom dye.
2. Select  .
3. Select **Yes** to confirm deletion.

**NOTICE** Deleting a custom dye permanently removes the dye and all associated information from the software.

## Detection Limits

The lower detection limits and reproducibility specifications for nucleic acids are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the user-defined extinction coefficients.

### To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

**Note** For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

### Related Topics

- Detection Limits for All Applications

# Calculations for Microarray Measurements

As with the other nucleic acid applications, the Microarray application uses a modification of the Beer-Lambert equation to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor." The Microarray application offers six options (shown at right) for selecting an appropriate factor for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the factor is known, choose the Custom Factor option and enter the factor in ng-cm/µL. Otherwise, choose the option that best matches the sample solution.

**Tip**: Ideally, the factor or extinction coefficient should be determined empirically using a solution of the study nucleic acid at a known concentration using the same buffer.

### Available Options for Factors

- **dsDNA** (factor = 50 ng-cm/µL)
- **ssDNA** (factor = 33 ng-cm/µL)
- **RNA** (factor = 40 ng-cm/µL)
- **Oligo DNA** (calculated from user entered DNA nucleotide sequence)
- **Oligo RNA** (calculated from user entered RNA nucleotide sequence)
- **Custom Factor** (user entered factor between 15 ng-cm/µL and 150 ng-cm/µL

**Note**: See Sample Type for more information.

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

### A260 absorbance

**Note**: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 nm is zero in the displayed spectra. Also, for micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Nucleic acid absorbance values for all Microarray sample types are measured at 260 nm using the 850-corrected and normalized spectrum.
- If Analysis Correction is selected, the absorbance value at the correction wavelength is subtracted from the absorbance at 260 nm.
- If one or more dyes are selected, the dye correction values at 260 nm are also subtracted from the absorbance at 260 nm.
- The final corrected absorbance at 260 nm is reported and used to calculate sample concentration.

### A280 absorbance

- 850-corrected and normalized absorbance value at 280 nm (minus the A280 dye correction) is used to calculate an A260/A280 ratio.

Dye concentrations are calculated from the absorbance value at the dye's analysis wavelength, the dye's extinction coefficient, and the sample pathlength.

### Dye absorbance

- Dye absorbance values are measured at specific wavelengths. See Dye/Chromophore Editor for analysis wavelengths used.
- Baseline-corrected dye absorbance values are reported and used to calculate dye concentrations.

### Dye correction

- Pre-defined dyes have known correction values for A260 and A280. See Dye/Chromophore Editor for correction values used.
- A260 dye corrections are subtracted from the A260 absorbance value used to calculate nucleic acid concentration, and from the A260 absorbance value used to calculate the A260/A280 purity ratio.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is used. (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

- **Nucleic acid concentration**. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~1.8 is generally accepted as "pure" for DNA (~2.0 for RNA). Acidic solutions may under represent the reported value by 0.2-0.3; the opposite is true for basic solutions.
- **Dye1/Dye2 concentration**. Reported in pmol/µL. Calculations are based on Beer's Law equation using baseline-corrected dye absorbance value(s).

**Note**: Although purity ratios are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream application of interest (e.g., microarray).

### Related Topics

- Calculations for Nucleic Acid Measurements

# Custom Factor

Measures the concentration of purified nucleic acids using a custom factor for the calculations.



## Measure Custom Factor

Use the Custom Factor application to quantify purified DNA or RNA samples that absorb at 260 nm with a user-defined extinction coefficient or factor. The application reports nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

### Procedure

1. From the home screen, select the **Nucleic Acids** tab, then select **Custom Factor**.

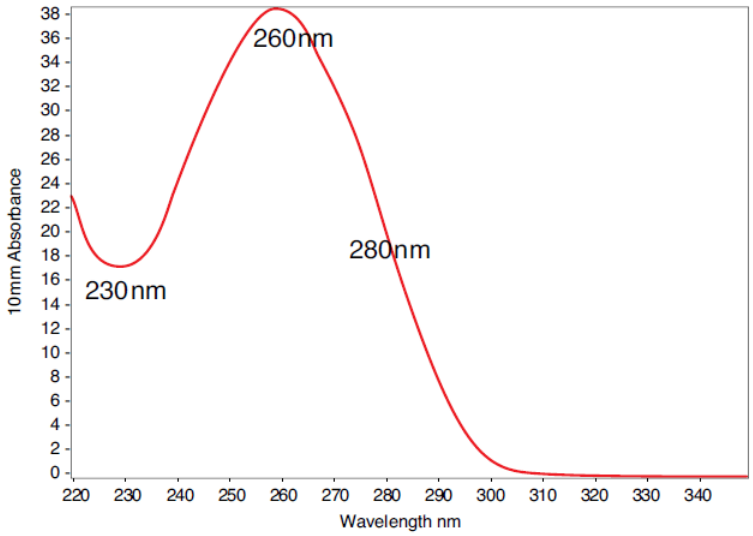
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
7. Pipette 1-2 µL sample solution onto the pedestal or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.
   - Cuvette: Select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**Typical nucleic acid spectrum**

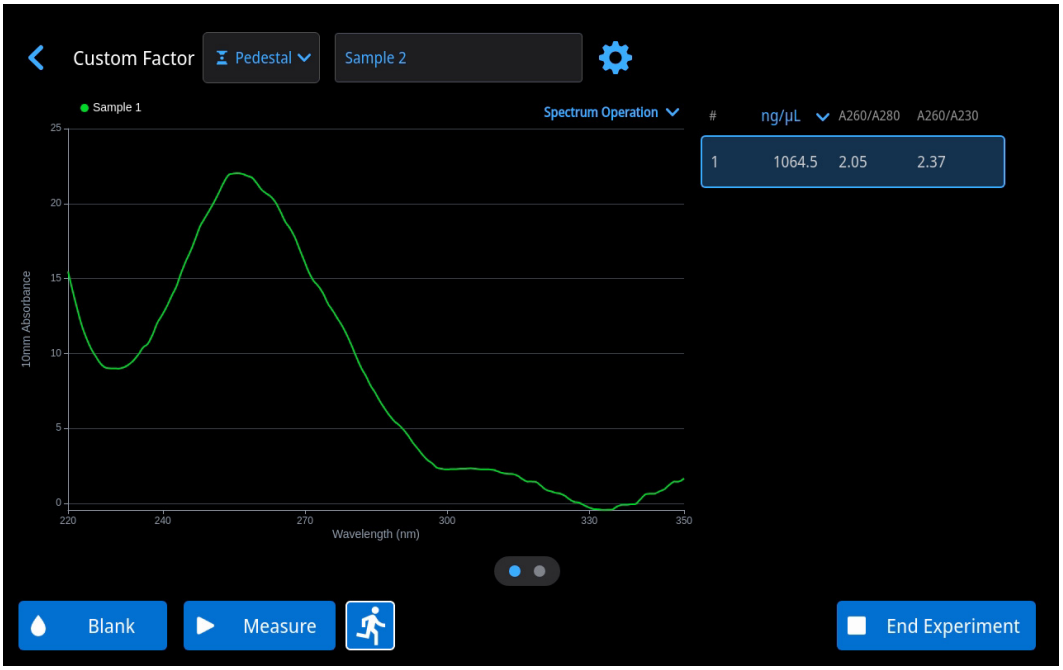
## Related Topics

- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Best Practices for Micro-Volume Measurements
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Custom Factor measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the measurement screen of the NanoDrop Ultra local control software:




**Note** The Custom Factor measurement screen is identical to the measurement screen for the other standard nucleic acid applications.

## Custom Factor measurement screen (PC control)

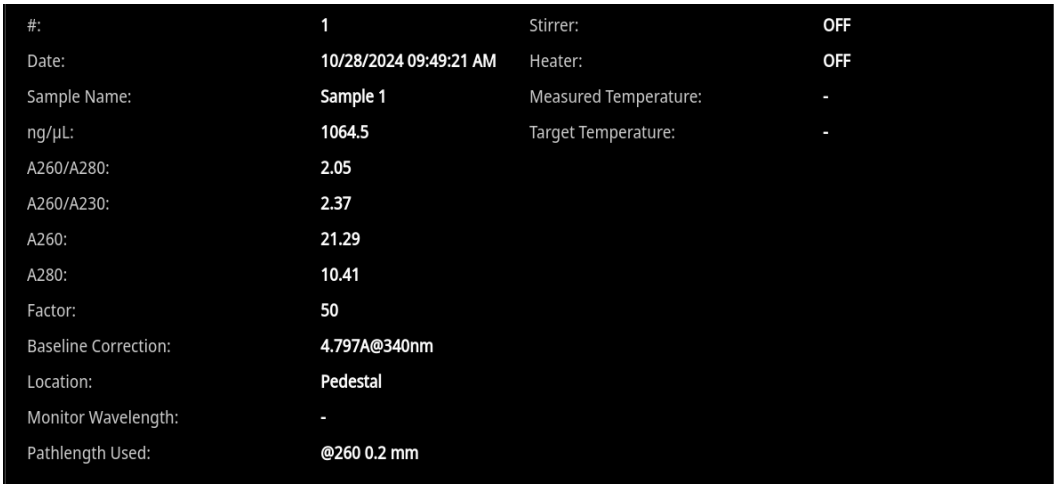
For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

### Related Topics

- Basic Instrument Operations
- Nucleic Acid Reported Results
- Nucleic Acid Calculations

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction
- location
- monitor wavelength
- pathlength used
- stirrer
- heater
- measured temperature
- target temperature

## Settings

The Custom Factor Setup screen appears after you select the Custom Factor application from the Nucleic Acids tab on the home screen. To show the Custom Factor settings, from the measurement screen, select Custom Factor Setup  .

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name "sample" followed by the number sample in the sequence. For example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name. |
| Custom Factor | Enter an integer value between 15 ng-cm/µL and 150 ng-cm/µL | Constant used to calculate nucleic acid concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength:<br><br>$f = 1/(\varepsilon_{260} * b))$<br><br>where:<br>**f**= factor<br>$\varepsilon$ = molar extinction coefficient at 260 nm in ng-cm/µL<br>**b** = sample pathlength in cm (1 cm for nucleic acids measured with the NanoDrop Ultra instruments) |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

### Related Topics

- Instrument Settings

## Detection Limits

The lower detection limits and reproducibility specifications for nucleic acids are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the user-defined extinction coefficients.

### To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

**Note** For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

### Related Topics

- Detection Limits for All Applications

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# Oligo DNA or Oligo RNA

Measures the concentration of purified ssDNA or RNA oligonucleotides that absorb at 260 nm.



## Measure Oligo DNA or Oligo RNA

Use the Oligo DNA and Oligo RNA applications to quantify oligonucleotides that absorb at 260 nm. Molar extinction coefficients are calculated automatically based on the user-defined base sequence of the sample. These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** If the oligonucleotide has been modified, for example with a fluorophore dye, check with the oligo manufacturer to determine if the modification contributes absorbance at 260 nm. If it does, we recommend using the Microarray application to quantify nucleic acid concentration. The Microarray application includes a correction to remove any absorbance contribution due to the dye from the oligo quantification result.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

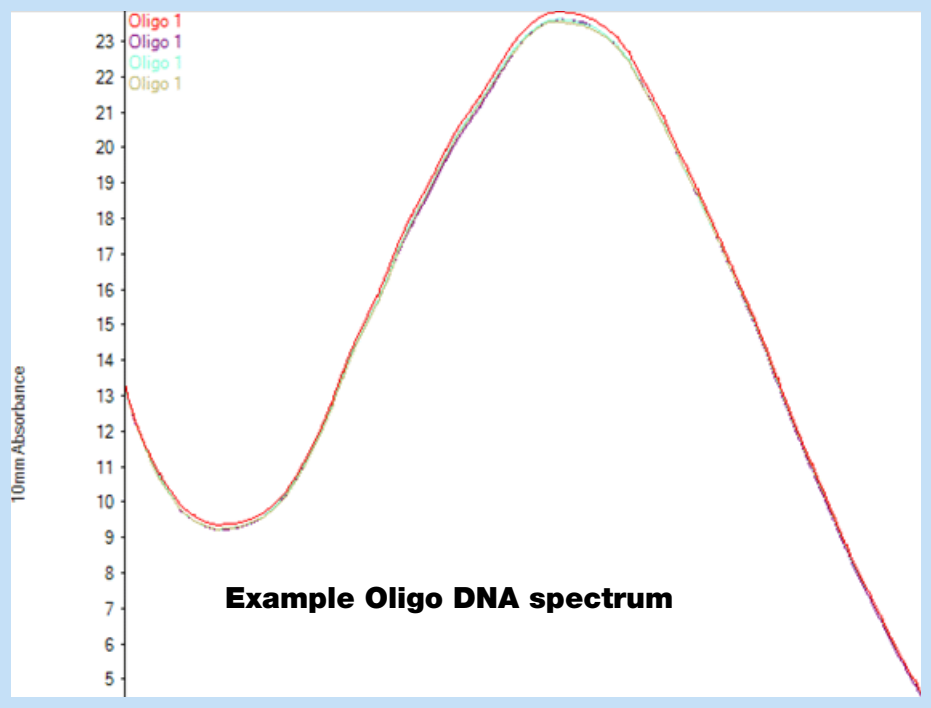
1. From the home screen, select the **Nucleic Acids** tab, then select either **Oligo DNA** or **Oligo RNA**, as needed.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
7. Pipette 1-2 µL sample solution onto the pedestal or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.
   - Cuvette: Select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**Example Oligo DNA spectrum**

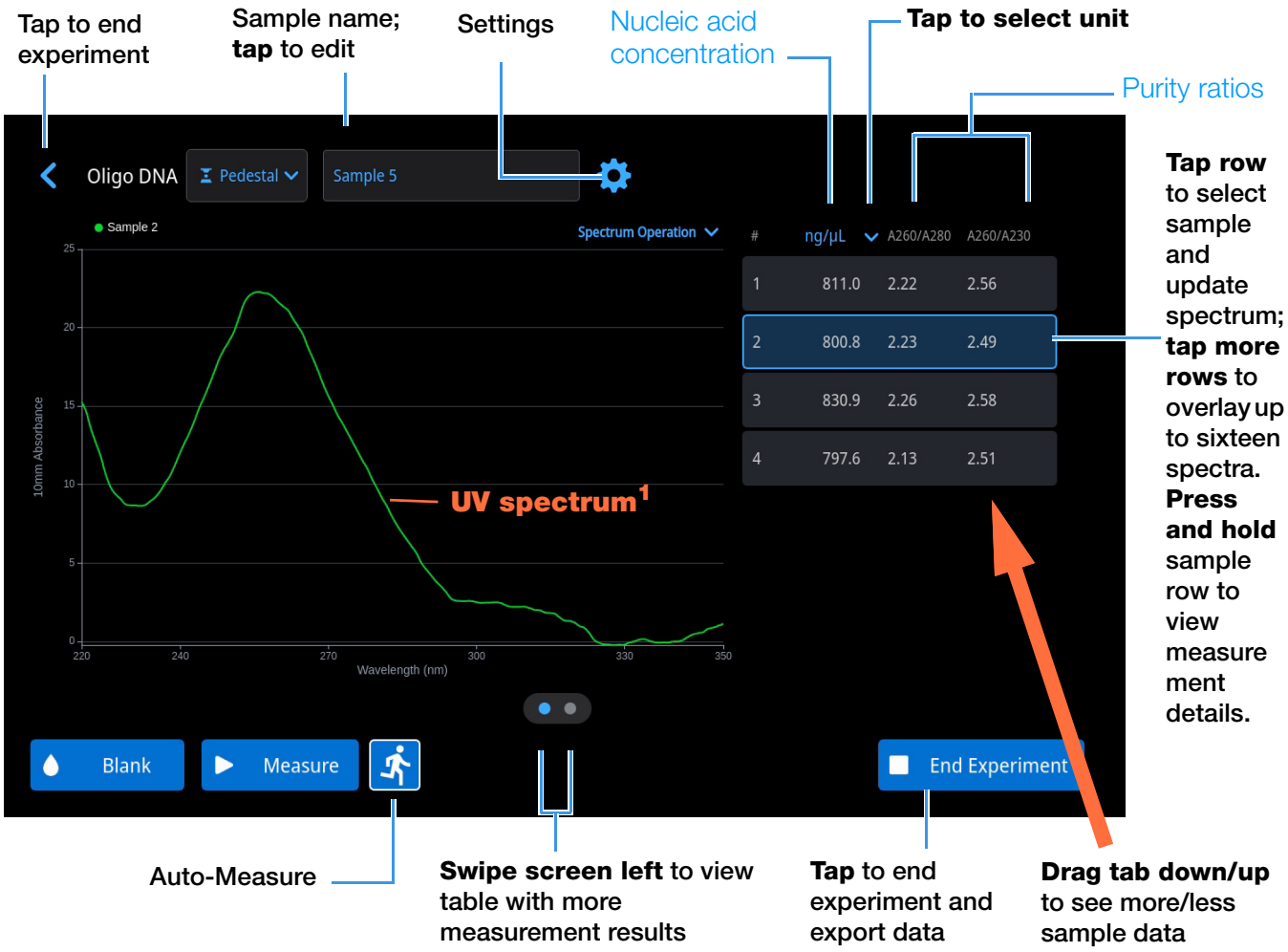
## Related Topics

- Best Practices for Nucleic Acid Measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Best Practices for Micro-Volume Measurements
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Oligo DNA measurement screen (local control)

For each measured sample, the Oligo DNA and Oligo RNA applications show the UV absorbance spectrum and a summary of the results. Here is an example of the measurement screen of the NanoDrop Ultra local control software:




[1]Measured oligo: TTT TTT TTT TTT TTT TTT TTT TTT

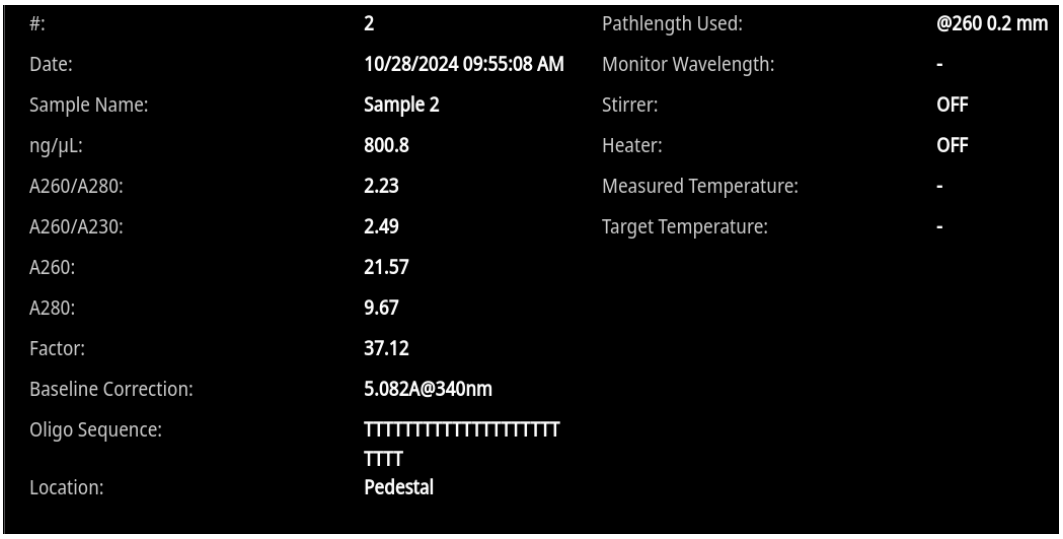
**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Oligo DNA or Oligo RNA measurement screen (PC control)

For each measured sample, the Oligo DNA and Oligo RNA applications show the UV absorbance spectrum reported results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



| | | | |
|---|---|---|---|
| #: | 2 | Pathlength Used: | @260 0.2 mm |
| Date: | 10/28/2024 09:55:08 AM | Monitor Wavelength: | - |
| Sample Name: | Sample 2 | Stirrer: | OFF |
| ng/µL: | 800.8 | Heater: | OFF |
| A260/A280: | 2.23 | Measured Temperature: | - |
| A260/A230: | 2.49 | Target Temperature: | - |
| A260: | 21.57 | | |
| A280: | 9.67 | | |
| Factor: | 37.12 | | |
| Baseline Correction: | 5.082A@340nm | | |
| Oligo Sequence: | TTTTTTTTTTTTTTTTTTTT | | |
| Location: | Pedestal | | |

- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction
- oligo sequence
- location

- pathlength used
- monitored wavelength
- stirrer
- heater
- measured temperature
- target temperature

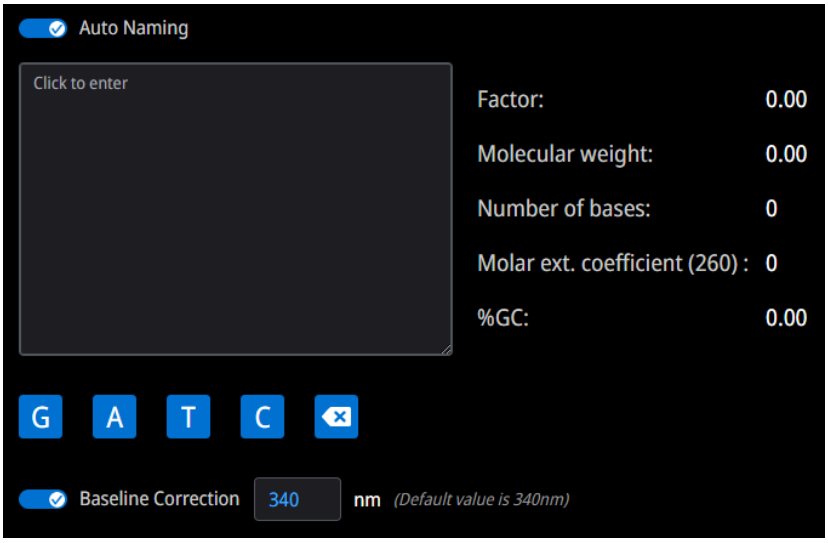
**Note** The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information.

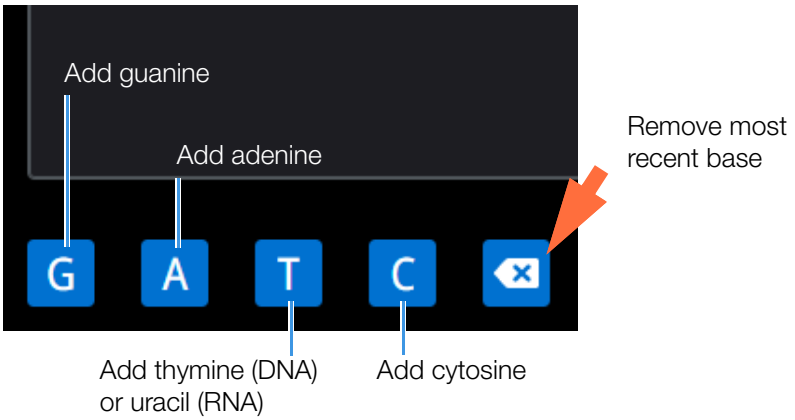
### Related Topics

- Basic Instrument Operations
- Oligo Calculations

## Settings

The Oligo Setup screen appears after you select the Oligo DNA or Oligo RNA application from the Nucleic Acids tab on the home screen. To show the Oligo settings from the Oligo measurement screen, select Oligo Setup .



| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name "sample" followed by the number sample in the sequence. For example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name. |
| Oligo Base Sequence | for DNA: Use the G, A, T and C keys to specify the DNA base sequence<br><br>for RNA: Use the G, A, U and C keys to specify the RNA base sequence | Specify your DNA or RNA base sequence.<br>Tap or click the corresponding keys:<br>  <br><br>From the PC control software, you can also enter base sequence using the keyboard, or by copy and pasting a sequence from another application. |
| | | Each time a base is added to the sequence, the software calculates the following:<br><br>• **Factor**. Constant used to calculate oligonucleotide concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength:<br><br>$\mathbf{f = 1/(\varepsilon_{260} * b)}$<br><br>where:<br>**f**= factor<br>$\varepsilon$ = molar extinction coefficient at 260 nm in ng-cm/µL<br>**b** = sample pathlength in cm (0.1 cm for nucleic acids measured with the NanoDrop Ultra instrument) |

| Setting | Available Options | Description |
|---|---|---|
| | | • **Molecular Weight** of oligo calculated from user-defined base sequence.<br>• **Number of Bases** entered.<br>• **Molar Ext. Coefficient (260 nm)**. Molar extinction coefficient of oligo (in ng-cm/µL) at 260 nm calculated from entered base sequence.<br>• **%GC**. Percentage of guanine and cytosine residues in total number of bases entered. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction. |

## Detection Limits

The lower detection limits and reproducibility specifications for the oligonucleotide sample types (ssDNA and RNA) are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the extinction coefficients for the user-defined base sequences.

### To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

**Note** For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

# Calculations

As with the other nucleic acid applications, the Oligo applications use the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength. Because oligonucleotides are short, single-stranded molecules (or longer molecules of repeating sequences), their spectrum and extinction coefficient ($\varepsilon$) are closely dependent on base composition and sequence.

(The generally accepted extinction coefficients and factors for single-stranded DNA and RNA provide a reasonable estimate for natural, essentially randomized, sequences but not for short, synthetic oligo sequences.) To ensure the most accurate results, we use the exact value of $\varepsilon_{260}$ to calculate oligonucleotide concentration.

The NanoDrop software allows you to specify the base sequence of an oligonucleotide before it is measured. For any entered base sequence, the software uses the equation at the right to calculate the extinction coefficient.

**Tip**: The extinction coefficient is wavelength specific for each oligonucleotide and can be affected by buffer type, ionic strength and pH.

## Extinction Coefficients for Oligonucleotides

The software uses the nearest neighbor method and the following formula to calculate molar extinction coefficients for specific oligonucleotide base sequences:

$$\varepsilon_{260} = \sum_{1}^{N-1} \varepsilon_1 - \sum_{2}^{N-1} \varepsilon_2 + \sum_{1}^{N} \varepsilon_3$$

where:
$\varepsilon$ = molar extinction coefficient in L/mole-cm
$\varepsilon_1 = \varepsilon_{\text{nearest neighbor}}$
$\varepsilon_2 = \varepsilon_{\text{individual bases}}$
$\varepsilon_3 = \varepsilon_{\text{modifications, such as fluorescent dyes}}$

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

### A260 absorbance

**Note**: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the sample absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

### A230, A280 absorbance

- Normalized absorbance values at 230 nm, 260 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. Estimated A260/A280 ratios for each independently measured nucleotide are provided below:

Guanine: 1.15
Adenine: 4.50
Cytosine: 1.51
Uracil: 4.00
Thymine: 1.47

The A260/A280 ratio for a specific nucleic acid sequence is approximately equal to the weighted average of the A260/A280 ratios for the four nucleotides present.

**Note**: RNA will typically have a higher 260/280 ratio due to the higher ratio of Uracil compared to that of Thymine.

## Reported Values

- **Nucleic acid concentration**. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm.
- **A260/A230 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm.

**Note**: The traditional purity ratios (A260/A280 and A260/A230), which are used as indicators of the presence of various contaminants in nucleic acid samples, do not apply for oligonucleotides because the shapes of their spectra are highly dependent on their base compositions. See side bar for more information.

## Related Topics

- Calculations for Nucleic Acid Measurements

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# Protein Applications

## Protein A280

Measures the concentration of purified protein samples that absorb at 280 nm.

Measure Protein A280

Reported Results

Settings

Protein Editor

Detection Limits

Calculations

### Best practices for protein measurements

- Isolate and purify protein samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA and some buffer components. See Preparing Samples for more information.

  **Note** Extraction reagents that contribute absorbance between 200 nm and 280 nm will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Choosing a blank:
  - For the Protein A280, Protein A205, and Proteins & Labels applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.

  - For the Protein BCA, Protein Bradford, and Protein Lowry applications, blank with deionized water (DI $H_2O$).
  - For the Protein Pierce 660 application, blank with the reference solution used to make the standard curve (reference solution should contain none of the standard protein stock). For more information, see Working with standard curves.

- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 280 nm or 205 nm), you may need to choose a different buffer or application, such as a colorimetric assay (for example, BCA or Pierce 660). See Choosing and Measuring a Blank for more information.

  **Note** Buffers such as Triton X, RIPA, and NDSB contribute significant absorbance and are not compatible with direct A280 or A205 measurements.

- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned. (Proteins tend to stick to pedestal surfaces.)
  - Gently (but thoroughly) vortex samples before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop Ultra$^C$ and NanoDrop Ultra$^C$ FL instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

## Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Measure Protein A280

Use the Protein A280 application to quantify purified protein samples that contain amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, which exhibit absorbance at 280 nm. This application reports protein concentration measured at 280 nm and one absorbance ratio (A260/A280). A single-point baseline correction can also be used. This application does not require a standard curve.

### Before you begin...

Before taking pedestal measurements with one of the NanoDrop Ultra instruments, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** If your samples contain mainly peptide bonds and little or no amino acids, use the Protein A205 application instead of Protein A280.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein A280**.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, from the drop-down menu at the top of the screen, select **Cuvette** to display the cuvette settings.
     Select the desired pathlength, stir speed, and heating, and then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.

5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.

7. Pipette 2 µL sample solution onto the pedestal, or insert the sample cuvette into the cuvette holder.

8. Start the sample measurement:

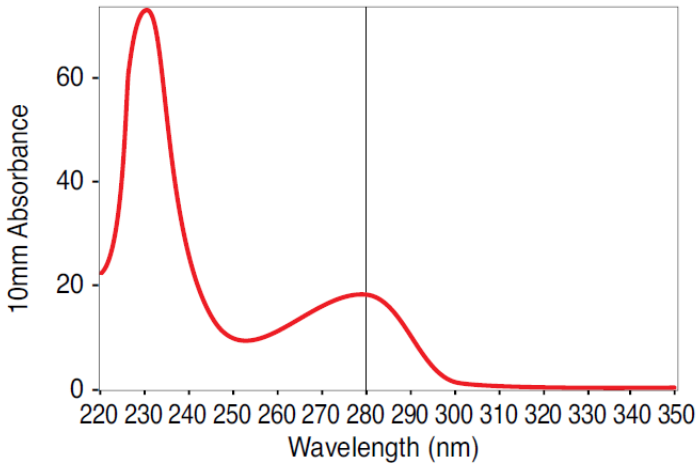
   – Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.

   – Cuvette: Select **Measure**.

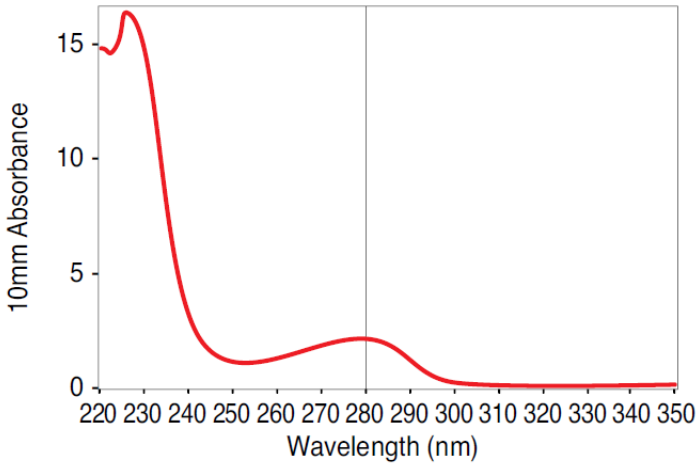
   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.

10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**High concentration BSA sample**

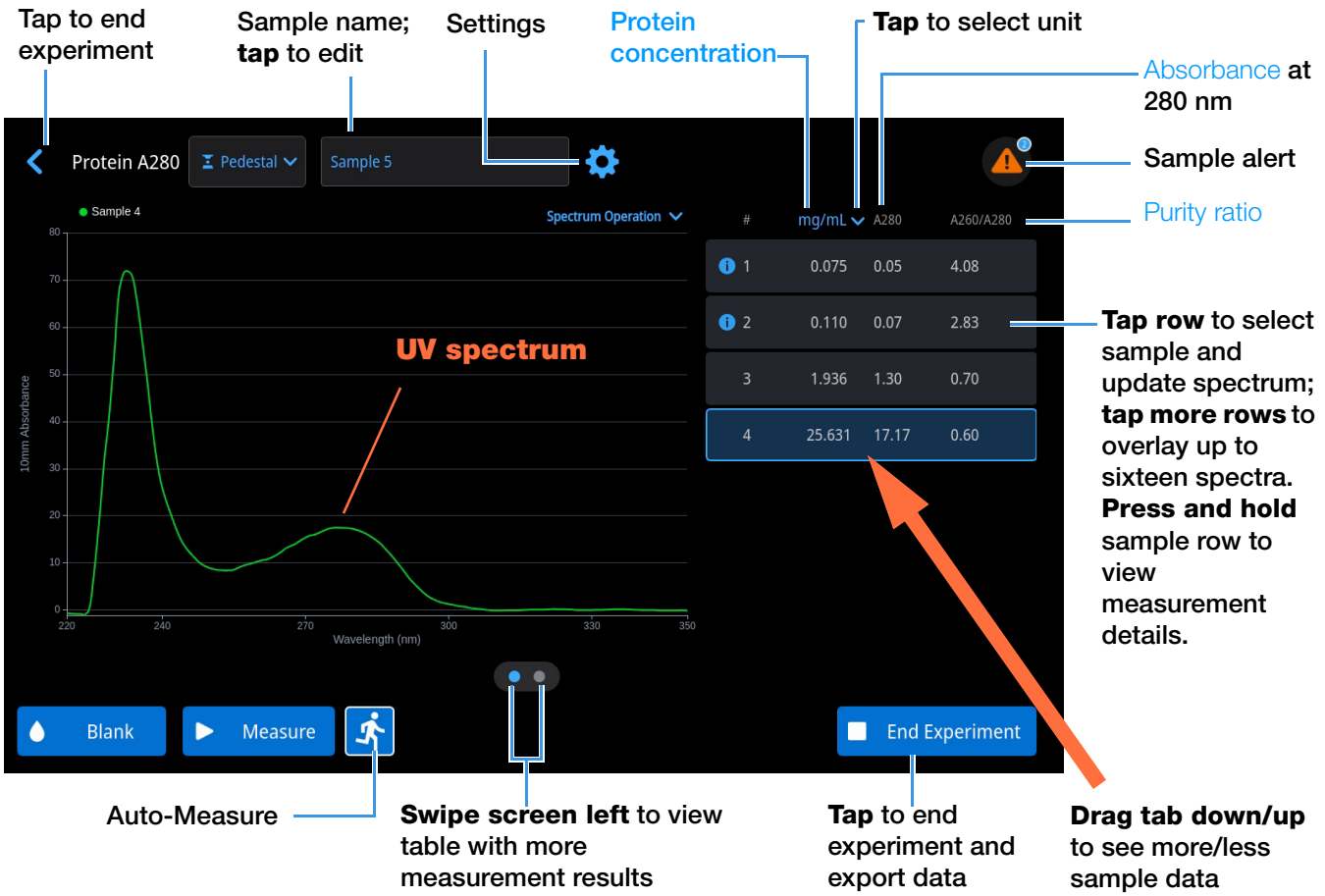



**Low concentration BSA sample**

# Reported Results

## Protein A280 measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The following is an example of the measurement screen of the NanoDrop Ultra local control software:



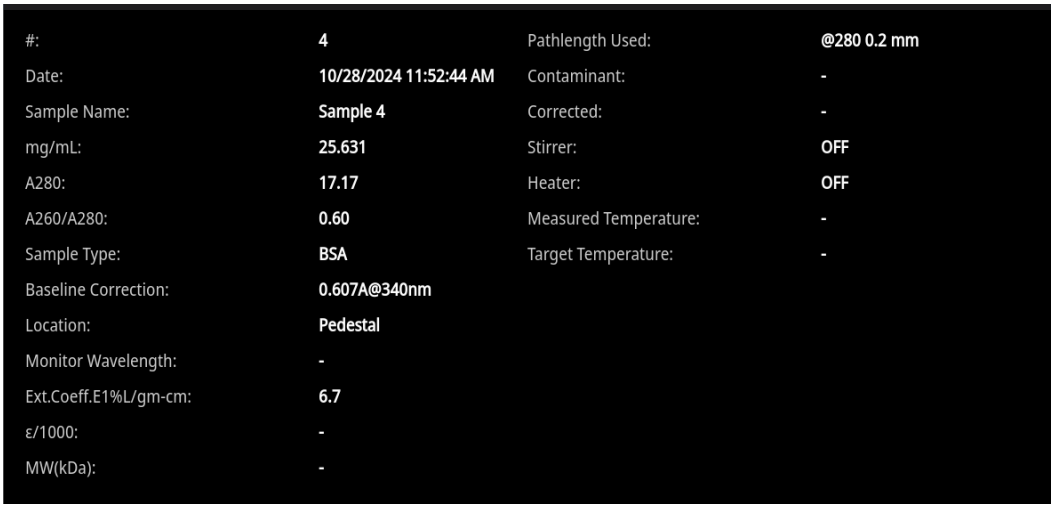

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Protein A280 measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



| | | | |
|---|---|---|---|
| #: | 4 | Pathlength Used: | @280 0.2 mm |
| Date: | 10/28/2024 11:52:44 AM | Contaminant: | - |
| Sample Name: | Sample 4 | Corrected: | - |
| mg/mL: | 25.631 | Stirrer: | OFF |
| A280: | 17.17 | Heater: | OFF |
| A260/A280: | 0.60 | Measured Temperature: | - |
| Sample Type: | BSA | Target Temperature: | - |
| Baseline Correction: | 0.607A@340nm | | |
| Location: | Pedestal | | |
| Monitor Wavelength: | - | | |
| Ext.Coeff.E1%L/gm-cm: | 6.7 | | |
| ε/1000: | - | | |
| MW(kDa): | - | | |

- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- protein concentration
- A280
- A260/A280
- sample type
- baseline correction
- location
- monitored wavelength
- mass extinction coefficient (1% solution)
- molar extinction coefficient
- molecular weight (kDA)
- pathlength used
- contaminant
- corrected protein concentration
- stirrer

- heater
- measured temperature
- target temperature

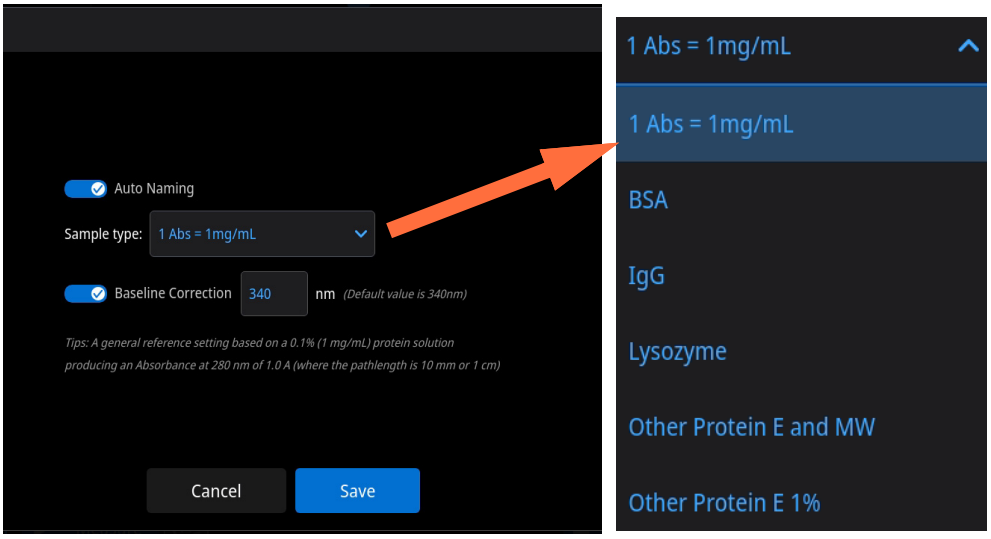
### Related Topics

- Basic Instrument Operations
- Protein A280 Calculations

## Settings

The Protein A280 Setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein A280 settings, from the Protein A280 measurement screen, select the setup icon .

The Protein A280 application provides a variety of sample type options for purified protein analysis.




Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the ε + MW (molar) or ε1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option.

**Tip** Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Auto Naming | On or Off | N/A | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | On or Off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | N/A | Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction. |
| Sample type[a] | 1 Abs = 1 mg/mL | General reference | Recommended when extinction coefficient is unknown and rough estimate of protein concentration is acceptable for a solution with no other interfering substances. Assumes 0.1% (1 mg/mL) protein solution produces 1.0A at 280 nm (where pathlength is 10 mm), i.e., $\varepsilon 1\% = 10$. |
| | BSA | 6.7 | Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient ($\varepsilon$) of 6.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) BSA solution. Assuming MW is 66,400 daltons (Da), molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$. |

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| | IgG | 13.7 | Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (ε) of 13.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) IgG solution. Assuming MW is 150,000 Da, molar extinction coefficient at 280 nm for IgG is approximately 210,000 $M^{-1}cm^{-1}$. |
| | Lysozyme | 26.4 | Calculates lysozyme protein concentration using mass extinction coefficient (ε) of 26.4 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) lysozyme solution. Assumes molar extinction coefficient for egg white lysozyme ranges between 36,000 $M^{-1}cm^{-1}$ and 39,000 $M^{-1}cm^{-1}$. |
| | Other protein (ε + MW) | User entered molar extinction coefficient and molecular weight | Assumes protein has known molar extinction coefficient (ε) and molecular weight (MW), where:<br>$(\varepsilon_{molar})*10=(\varepsilon_{percent})*(MW_{protein})$<br>Enter MW in kiloDaltons (kDa) and molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (i.e., ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210. |
| | Other protein (ε1%) | User entered mass extinction coefficient | Assumes protein has known mass extinction coefficient (ε). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (ε1%) protein solution. |

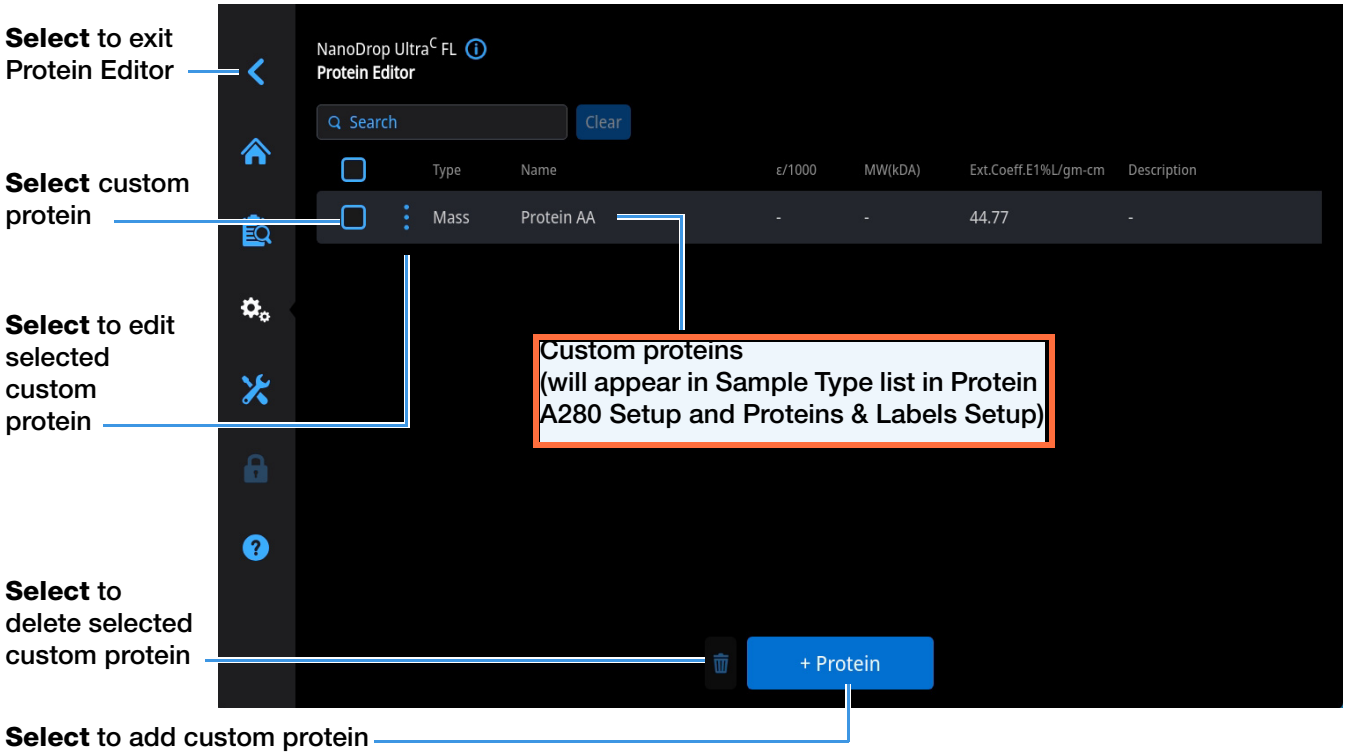
[a] To add or edit a custom protein, use Protein Editor.

# Protein editor

Use the Protein Editor to add a custom protein to the list of available protein sample types in Protein A280 Setup and Proteins & Labels Setup.

### To access the Protein Editor:

- From the software home screen, select **Settings > Protein Editor**.

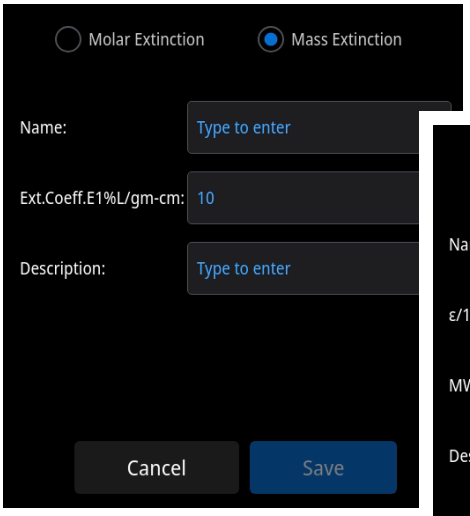



**Protein Editor as seen on the NanoDrop Ultra local control software**

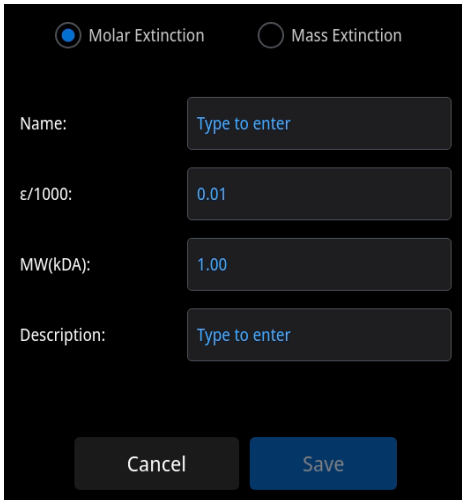
These operations are available from the Protein Editor:

### Add custom protein

1. In Protein Editor, select + Protein to show the Add Protein box.
2. Enter a unique **Name** for the new protein (from local control, tap field to display keyboard, tap **Done** key to close keyboard).
3. Enter a **Description** for the new protein.
4. Specify whether to enter **Molar Extinction** coefficient or **Mass Extinction** coefficient for the custom protein by selecting the appropriate radio button.
   - If **Mass Extinction** coefficient is selected, enter mass extinction coefficient in L/gm-cm for 10 mg/mL (Ɛ1%) protein solution.




**Select** a field to show keyboard (local control only); to close, select **Save**




- If **Molar Extinction** is selected,
  - Enter molar extinction coefficient (Ɛ) in $M^{-1}cm^{-1}$ divided by 1000 (that is, Ɛ/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.
  - Enter molecular weight (MW) in kiloDaltons (kDa).

5. Select **Save** to close the **Add Protein** box.

   The new custom protein appears in the **Sample Type** list in Protein A280 Setup, Proteins & Labels Setup, and Protein A280 Pro Setup.

### Edit custom protein

1. In Protein Editor, select ⋮ in the row of the desired Protein type that needs to be edited.
2. Select Edit to show the **Edit Protein** box.
3. Edit any entries or settings.
4. Select **Save**.

### Delete custom protein

1. In Protein Editor, select one or more custom proteins to delete by using the check boxes to the left of each desired Protein.
2. Select 🗑.

3. Confirm deletion of the custom protein(s) by selecting **Yes**.

**Note** Deleting a custom protein permanently removes the protein and all associated information from the software.

## Detection Limits

Detection limits and reproducibility specifications for purified BSA proteins are provided here. The BSA lower detection limit and reproducibility values apply to any protein sample type. The upper detection limits are dependent on the upper absorbance limit of the instrument and the sample's extinction coefficient.

### To calculate upper detection limits for other (non-BSA) protein sample types

To calculate upper detection limits in ng/µL for proteins, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} / \text{mass extinction coefficient}_{\text{sample}}) * 10$$

For example, if the sample's mass extinction coefficient at 280 nm is 6.7 for a 1% (10 mg/mL) solution, the equation looks like this:

(550 / 6.7) * 10 = 824.6 (or ~825)

## Calculations

The Protein A280 application uses the Beer-Lambert equation to correlate absorbance with concentration. Solving Beer's law for concentration yields the equation at the right.

**Beer-Lambert Equation (solved for concentration)**

$$c = A / (\varepsilon * b)$$

where:

A = UV absorbance in absorbance units (AU)

$\varepsilon$ = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) in liter/mol-cm

b = pathlength in cm

c = analyte concentration in moles/liter or molarity (M)

**Note**: Dividing the measured absorbance of a sample solution by its molar extinction coefficient yields the molar concentration of the sample. See Published Extinction Coefficients for more information regarding molar vs. mass concentration values.

The extinction coefficient of a peptide or protein is related to its tryptophan (W), tyrosin (Y) and cysteine (C) amino acid composition.

**Tip**: The extinction coefficient is wavelength specific for each protein and can be affected by buffer type, ionic strength and pH.

This application offers six options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the ε + MW (molar) or ε1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

**Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

## Extinction Coefficients for Proteins

At 280 nm, the extinction coefficient is approximated by the weighted sum of the 280 nm molar extinction coefficients of the three constituent amino acids, as described in this equation:

$$\varepsilon = (nW * 5500) + (nY * 1490) + (nC * 125)$$

where:
ε = molar extinction coefficient
n = number of each amino acid residue
5500, 1490 and 125 = amino acid molar absorptivities at 280 nm

## Available Options for Extinction Coefficient

- **1 Abs = 1 mg/mL**, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- **BSA** (Bovine Serum Albumin, 6.7 L/gm-cm)
- **IgG** (any mammalian antibody, 13.7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26.4 L/gm-cm)
- **Other protein** (ε + MW), user-specified molar ext. coefficient
- **Other protein** (ε1%), user-specified mass ext. coefficient

**Note**: See Sample Type for details.

Most sources report extinction coefficients for proteins measured at or near 280 nm in phosphate or other physiologic buffer. These values provide sufficient accuracy for routine assessments of protein concentration.

## Published Extinction Coefficients

Published extinction coefficients for proteins may be reported as:

- wavelength-dependent molar absorptivity (or extinction) coefficient ($\varepsilon$) with units of $M^{-1}cm^{-1}$
- percent solution extinction coefficient ($\varepsilon$1%) with units of $(g/100\ mL)^{-1}cm^{-1}$ (i.e., 1% or 1 g/100 mL solution measured in a 1 cm cuvette)
- protein absorbance values for 0.1% (i.e., 1 mg/mL) solutions

**Tip**: Assess published values carefully to ensure unit of measure is applied correctly.

The equation at the right shows the relationship between molar extinction coefficient ($\varepsilon_{molar}$) and percent extinction coefficient ($\varepsilon$1%).

## Conversions Between $\varepsilon_{molar}$ and $\varepsilon$1%

$$(\varepsilon_{molar}) * 10 = (\varepsilon 1\%) * (MW_{protein})$$

Example: To determine percent solution extinction coefficient ($\varepsilon$1%) for a protein that has a molar extinction coefficient of 43,824 $M^{-1}cm^{-1}$ and a molecular weight (MW) of 66,400 daltons (Da), rearrange and solve the above equation as follows:

$$\varepsilon 1\% = (\varepsilon_{molar} * 10) / (MW_{protein})$$

$$\varepsilon 1\% = (43{,}824 * 10) / 66{,}400\ Da)$$

$$\varepsilon 1\% = 6.6\ g/100\ mL$$

To determine concentration (c) of a sample in mg/mL, use the equation at the right and a conversion factor of 10.

**Tip**: The NanoDrop Ultra software includes the conversion factor when reporting protein concentrations.

## Conversions Between g/100 mL and mg/mL

$$C_{protein}\ in\ mg/mL = (A / \varepsilon 1\%) * 10$$

Example: If measured absorbance for a protein sample at 280 nm relative to the reference is 5.8 A, protein concentration can be calculated as:

$$C_{protein} = (A / \varepsilon 1\%) * 10$$

$$C_{protein} = (5.8/6.6\ g/100\ mL) * 10$$

$$C_{protein} = 8.79\ mg/mL$$

Calculated protein concentrations are based on the absorbance value at 280 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction (or analysis correction) may be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm and 280 nm are used to calculate purity ratios for the measured protein samples.

Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

### A280 absorbance

**Note**: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Protein absorbance values are measured at 280 nm using the normalized spectrum. If Baseline Correction is not selected, this is the reported A280 value and the value used to calculate protein concentration.
- If Baseline Correction is selected, the normalized and baseline-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

### A260 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at 260 nm is used to calculate A260/A280 ratios.

## Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized. (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~0.57 is generally accepted as “pure” for proteins.

**Note**: Although purity ratios are important indicators of sample quality, the best indicator of protein quality is functionality in the downstream application of interest (e.g., real-time PCR).

- **Sample type**. Determines the extinction coefficient used in conjunction with Beer's Law to calculate sample concentration.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays whether the measurement was taken from the pedestal or cuvette mode.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.
- **Mass extinction coefficient (1% solution)**.
- **Molar extinction coefficient**.
- **Molecular weight**.
- **Contaminant**. If a contaminant was identified by the Acclaro software, the contaminant will be displayed in this column.
- **Corrected**. Displays the corrected analyte concentration determined using the Acclaro software, if one is available.
- **Stirrer**. "Off" will be displayed when the stirrer function on a cuvette model is not used. When the stirrer function is used, the stir speed is displayed.

- **Heater**. "Off" or "On" will be displayed to show if the cuvette port was heated during the measurement.
- **Measured Temperature**. Temperature of the cuvette port during the measurement will be displayed.
- **Target Temperature**. Desired temperature of the cuvette port will be displayed.

# Protein A205

Measures the concentration of purified protein populations that absorb at 205 nm.



## Measure Protein A205

Use the Protein A205 application to quantify purified peptides and other proteins that contain peptide bonds, which exhibit absorbance at 205 nm. This application reports protein concentration and two absorbance values (A205 and A280). A single-point baseline correction can also be used. This application does not require a standard curve.

**Before you begin...**

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** If your samples contain mainly amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, use the Protein A280 application instead of Protein A205.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

### Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein A205**.

2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL of the blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
7. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.
   - Cuvette: Select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
9. When you are finished measuring samples, tap **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

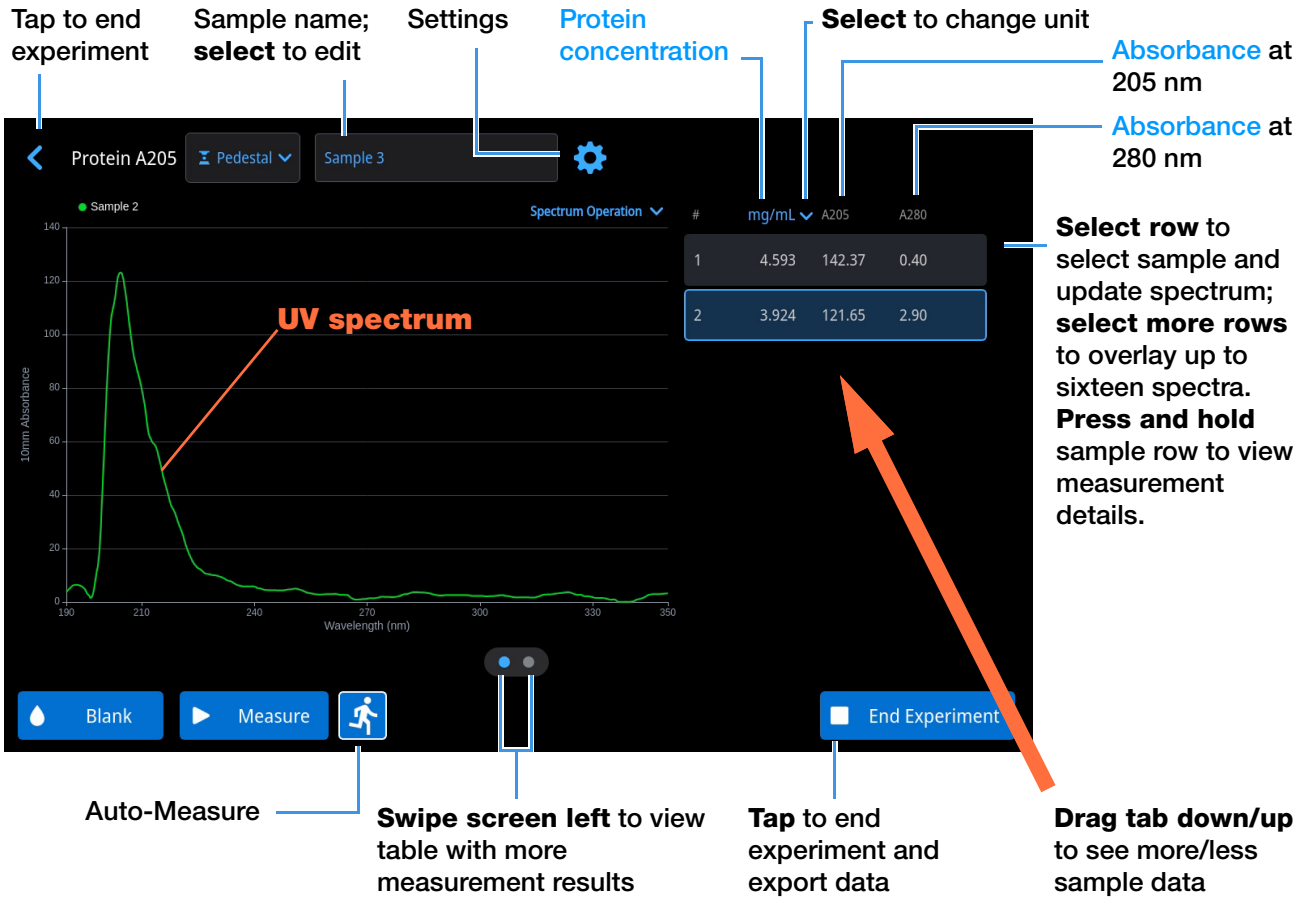
## Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Protein A205 Reported Results

## Protein A205 measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The following is an example of the measurement screen of the NanoDrop Ultra local control software:



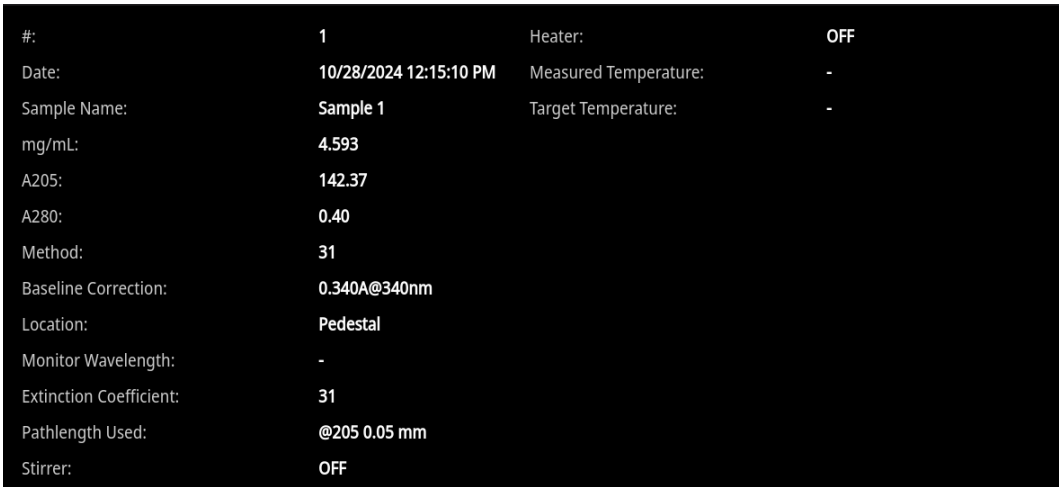

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Protein A205 measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Protein A205 reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



| | | | |
|---|---|---|---|
| #: | 1 | Heater: | OFF |
| Date: | 10/28/2024 12:15:10 PM | Measured Temperature: | - |
| Sample Name: | Sample 1 | Target Temperature: | - |
| mg/mL: | 4.593 | | |
| A205: | 142.37 | | |
| A280: | 0.40 | | |
| Method: | 31 | | |
| Baseline Correction: | 0.340A@340nm | | |
| Location: | Pedestal | | |
| Monitor Wavelength: | - | | |
| Extinction Coefficient: | 31 | | |
| Pathlength Used: | @205 0.05 mm | | |
| Stirrer: | OFF | | |

- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- protein concentration
- A205
- A280
- method
- baseline correction
- location
- monitored wavelength
- mass extinction coefficient (1% solution)
- pathlength used
- stirrer
- heater
- measured temperature
- target temperature

## Related Topics

- Basic Instrument Operations
- Protein A205 Calculations

# Settings

The Protein A205 Setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein A205 settings, from the Protein A205 measurement screen, select the setup icon .

The Protein A205 application provides a variety of method options for protein analysis.


| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Method | 31 | 31 | Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 31 |
| | Scopes | 27 + 120 * (A280/A205) | Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205) |
| | Other protein (Ɛ1%) | User entered mass extinction coefficient | Assumes protein has known mass extinction coefficient (Ɛ). Enter mass extinction coefficient in L/gm-cm for 1 mg/mL (Ɛ0.1%) protein solution. |

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Auto Naming | On or Off | N/A | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | On or Off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | N/A | Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction. |

## Detection Limits

See “Detection Limits for All Applications” on page 31.

# Calculations

As with the other protein applications, Proteins A205 uses the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength.

This application offers three options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the Ɛ1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

**Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

Calculated protein concentrations are based on the absorbance value at 205 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on the sample sequence.

### Available Options for Extinction Coefficient

- **31**, assumes Ɛ0.1% (1 mg/mL) at 205 nm = 31
- **Scopes**, assumes Ɛ0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
- **Other protein**, enter mass extinction coefficient in L/gm-cm for 1 mg/mL (Ɛ0.1%) protein solution

**Note**: See Sample Type for details.

### Measured Values

### A205 absorbance

**Note**: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Protein absorbance values are measured at 205 nm using the normalized spectrum. If Baseline Correction is not selected, this is the reported A205 value and the value used to calculate protein concentration.
- If Baseline Correction is selected, the normalized and baseline-corrected absorbance value at 205 nm is reported and used to calculate protein concentration.

### A280 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at 280 nm is also reported.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

- **Protein concentration**. Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **Method**. Determines the extinction coefficient used in conjunction with Beer's Law to calculate sample concentration.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays whether the measurement was taken from the pedestal or cuvette mode.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.
- **Mass extinction coefficient (1% solution)**.
- **Stirrer**. "Off" will be displayed when the stirrer function on a cuvette model is not used. When the stirrer function is used, the stir speed is displayed.
- **Heater**. "Off" or "On" will be displayed to show if the cuvette port was heated during the measurement.
- **Measured Temperature**. Temperature of the cuvette port during the measurement will be displayed.
- **Target Temperature**. Desired temperature of the cuvette port will be displayed.

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# Proteins and Labels

Measures the concentration of purified proteins that have been labeled with up to two fluorescent dyes.



## Measure Proteins and Labels

Use the Proteins and Labels application to quantify proteins and fluorescent dyes for protein array conjugates, as well as metalloproteins such as hemoglobin, using wavelength ratios. This application reports protein concentration measured at 280 nm, an A260/A280 absorbance ratio, and the concentrations and measured absorbance values of the dyes, allowing detection of dye concentrations as low as 0.2 picomole per microliter. This information is useful for evaluating protein/dye conjugation (degree of labeling) for use in downstream applications.

**Before you begin...**

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

### Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein & Labels**.

2. Configure any of the setup options if desired, then select **Save**.


   **Tip**: Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.

3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.

4. Pipette 1–2 µL of the blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.

5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

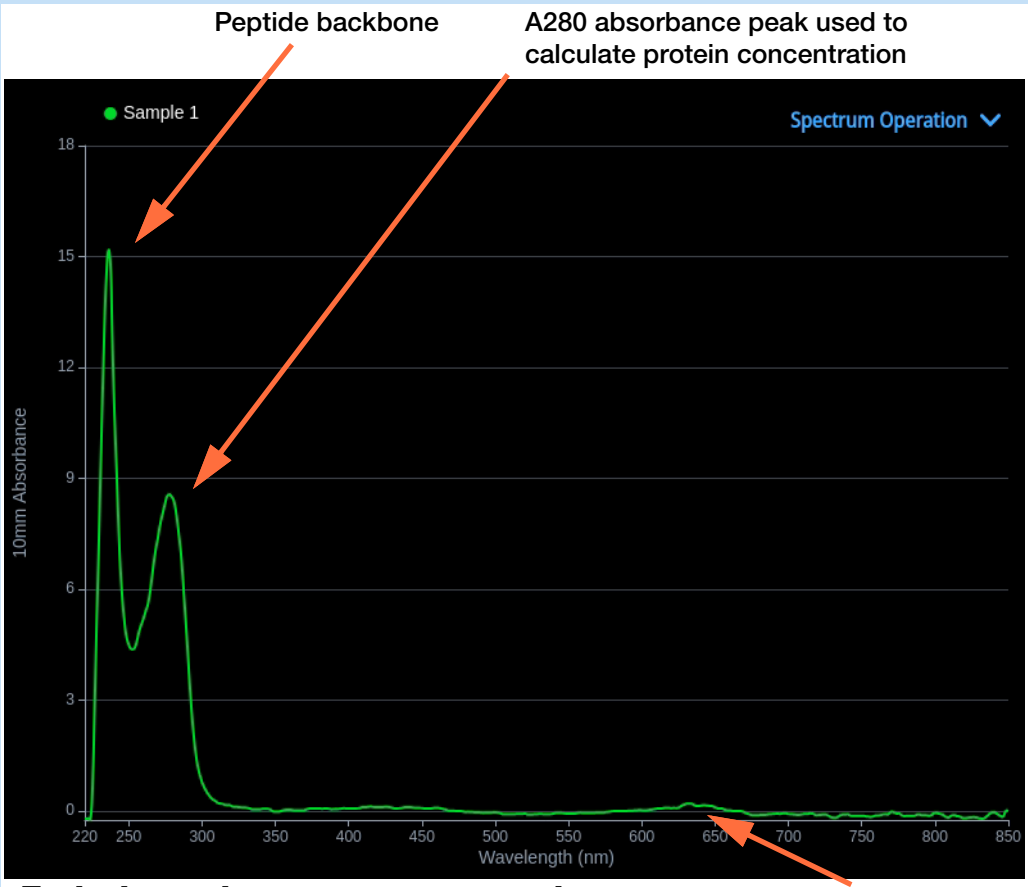
6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.

7. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.

8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is Off, lower arm and select **Measure**.
   - Cuvette: select **Measure**.

When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.




**Typical sample spectrum measured with Proteins & Labels application**

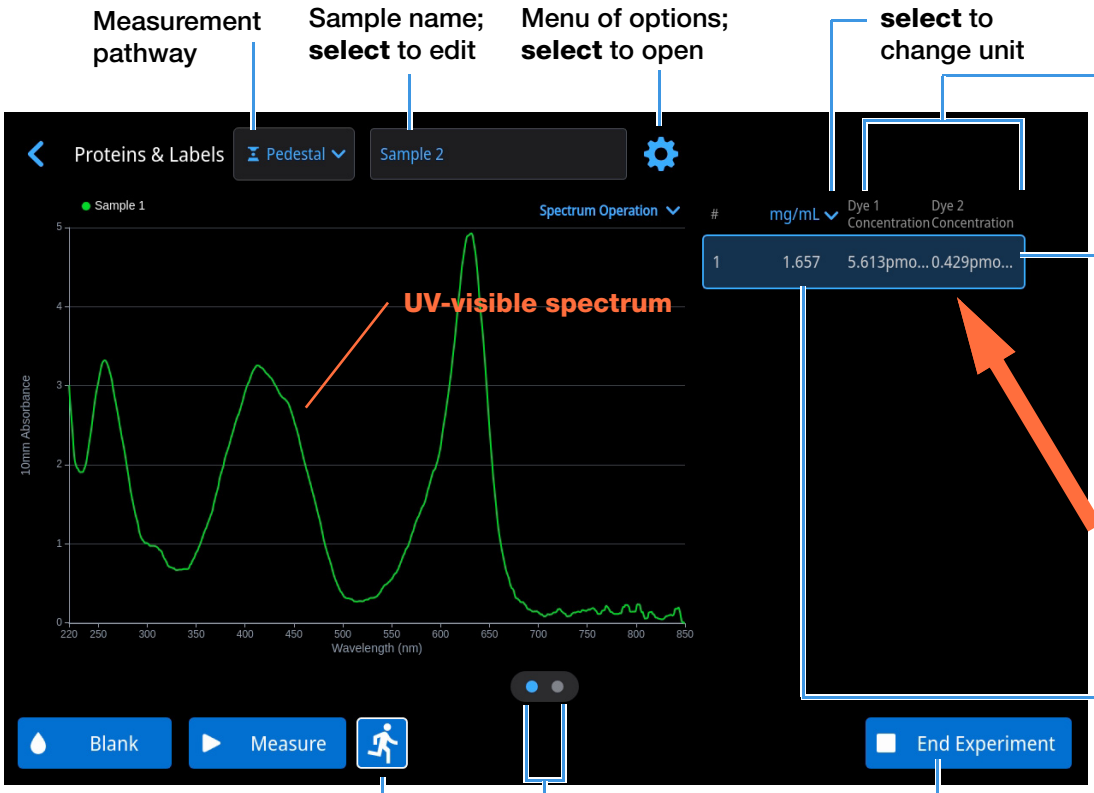
## Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Proteins & Labels measurement screen (local control).

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Below is an example of the measurement screen of the NanoDrop Ultra local control software:




**Note**

- A baseline correction is performed at 850 nm (absorbance value at 850 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Proteins & Labels measurement screen (PC control).

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

| | | | |
|---|---|---|---|
| #: | 1 | Dy2 DoL: | 0.164 |
| Date: | 10/28/2024 12:29:53 PM | Sample Type: | BSA |
| Sample Name: | Sample 1 | Sloping Dye Correction 400 - 850 nm: | ON |
| mg/mL: | 1.657 | Analysis correction: | 0.681A@340nm |
| A280: | 1.92 | Location: | Pedestal |
| A260/A280: | 1.69 | MW(kDa): | - |
| Dye1: | Alexa Fluor 647 | ε/1000: | - |
| Dye 1 Concentration: | 5.613pmol/µL | Ext.Coeff.E1%L/gm-cm: | 6.7 |
| Dye 1 Absorbance: | 1.52 | Pathlength Used: | 1 mm |
| Dy1 DoL: | 0.217 | Monitor Wavelength: | - |
| Dye2: | Alexa Fluor 660 | Stirrer: | OFF |
| Dye 2 Concentration: | 0.429pmol/µL | Heater: | OFF |
| Dye 2 Absorbance: | 0.06 | Measured Temperature: | - |

### Reported values for Proteins & Labels application

- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- protein concentration
- location
- A280
- A260/A280
- Dye name, concentration, and absorbance
- degree of labeling
- Monitored wavelength
- Pathlength used

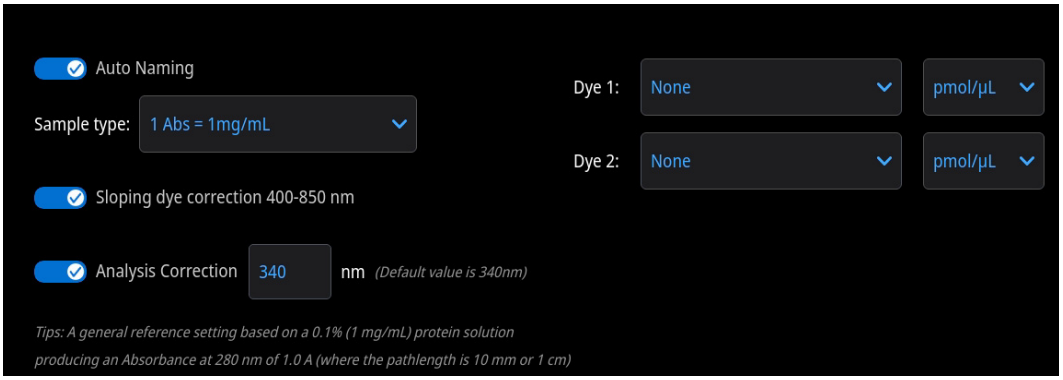
- mass extinction coefficient (1% solution)
- molar extinction coefficient
- molecular weight (kDA)
- sample type
- Analysis Correction
- Sloping dye correction
- stirrer
- heater
- measured temperature
- target temperature

### Related Topics

- Basic Instrument Operations
- Proteins & Labels calculations

## Settings

The Protein & Labels Setup screen appears after you select the application from the Proteins tab on the home screen. To show the Proteins & Labels settings from the Proteins & Labels measurement screen, select .



| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Sample type[a] | 1 Abs = 1 mg/mL | General reference | Select **Sample type** for detailed description of each available setting.<br><br>Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the Ɛ + MW (molar) or Ɛ1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option.<br><br>**Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer. |
| | BSA | 6.7 | |
| | IgG | 13.7 | |
| | Lysozyme | 26.4 | |
| | Other protein (Ɛ + MW) | user-entered molar extinction coefficient/ molecular weight | |
| | Other protein (Ɛ1%) | User entered mass extinction coefficient | |
| Analysis Correction[b] | On or Off<br><br>Enter analysis correction wavelength in nm or use default value (340 nm) | N/A | Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction. |
| Auto Naming | On or Off | N/A | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Dye 1/Dye 2 Type[c] | Cy3, 5, 3.5, or 5.5, Alexa Fluor 488, 546, 555, 594, 647, or 660 | See Dye/Chromophore Editor for specific values for each dye | Select pre-defined dye used to label sample material, or one that has been added using Dye/Chrom. Editor. |
| Dye 1/Dye 2 Unit | picomoles/microliter (pmol/µL), micromoles (uM), or millimoles (mM) | not applicable | Select unit for reporting dye concentrations. |
| Sloping Dye Correction[d] | On or Off | | Corrects dye absorbance measurements for any offset caused by light scattering particulates by subtracting absorbance value of a sloping baseline from 400 nm to 850 nm from absorbance value at dye's analysis wavelength. |

[a] To add or edit a custom protein, use Protein Editor.

[b] Analysis Correction affects calculation for protein concentration only.

[c] To add custom dye or edit list of available dyes, use Dye/Chromophore Editor.

[d] Sloping Dye Correction affects calculations for dye concentration only.

## Related Topics

- Instrument Settings
- Protein Editor
- Dye/Chromophore Editor

# Detection Limits

Detection limits and reproducibility specifications for purified BSA proteins and dyes that are pre-defined in the software are provided here. The BSA lower detection limit and reproducibility values apply to any protein sample type. The upper detection limits are dependent on the upper absorbance limit of the instrument and the sample's extinction coefficient.

### To calculate upper detection limits for other (non-BSA) protein sample types

To calculate upper detection limits in mg/mL for proteins, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} / \text{mass extinction coefficient}_{\text{sample}}) * 10$$

For example, if the sample's mass extinction coefficient at 280 nm is 6.7 for a 1% (10 mg/mL) solution, the equation looks like this:

$$(550 / 6.7) * 10 = 820.9 \text{ (or } \sim 820)$$

### Related Topics

- Detection Limits for All Applications

# Calculations

As with the other protein applications, Proteins & Labels uses the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength.

This application offers six options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the $\varepsilon$ + MW (molar) or $\varepsilon$1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

**Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

### Available Options for Extinction Coefficient

- **1 Abs = 1 mg/mL**, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- **BSA** (Bovine Serum Albumin, 6.7 L/gm-cm)
- **IgG** (any mammalian antibody, 13.7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26.4 L/gm-cm)
- **Other protein** ($\varepsilon$ + MW), user-specified molar ext. coefficient
- **Other protein** ($\varepsilon$1%), user-specified mass ext. coefficient

**Note**: See Sample Type for details.

Calculated protein concentrations are based on the absorbance value at 280 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction (or analysis correction) may be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

## Measured Values

### A280 absorbance

**Note**: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 nm is zero in the displayed spectra. Also, for micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Protein absorbance values are measured at 280 nm using the 850 nm-corrected and normalized spectrum. If Analysis Correction and Dye Correction are not selected, this is the reported A280 value and the value used to calculate protein concentration.
- If Analysis Correction is selected, the 850-corrected, normalized and analysis-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.
- If a Dye is used, the 850-corrected, normalized, analysis-corrected and dye-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

Dye concentrations are calculated from the absorbance value at the dye's analysis wavelength, the dye's extinction coefficient, and the sample pathlength. A sloped-line dye correction may also be used.

### Dye absorbance

- Dye absorbance values are measured at specific wavelengths. See Dye/Chromophore Editor for analysis wavelengths used.
- If Sloping Dye Correction is selected, a linear baseline is drawn between 400 nm and 850 nm and, for each dye, the absorbance value of the sloping baseline is subtracted from the absorbance value at each dye's analysis wavelength. Baseline-corrected dye absorbance values are reported and used to calculate dye concentrations.

### Dye correction

- Pre-defined dyes have known correction values for A260 and A280. See Dye/Chromophore Editor for correction values used.
- A280 dye correction is subtracted from A280 absorbance value used to calculate protein concentration.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

- **Location**. Displays whether the measurement was taken from the pedestal.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.
- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **Dye1/Dye2 concentration**. Reported in pmol/µL. Calculations are based on Beer's Law equation using (sloping) baseline-corrected dye absorbance value(s).
- **Dye1/Dye 2 degree of labeling**. Specifies the average number of fluorophore molecules per molecule of conjugate.
- **Stirrer**. "Off" will be displayed when the stirrer function on a cuvette model is not used. When the stirrer function is used, the stir speed is displayed.
- **Heater**. "Off" or "On" will be displayed to show if the cuvette port was heated during the measurement.
- **Measured Temperature**. Temperature of the cuvette port during the measurement will be displayed.
- **Target Temperature**. Desired temperature of the cuvette port will be displayed.

## Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

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# Protein BCA

Measures total protein concentration of unpurified protein samples using a bicinchoninic acid colorimetric detection reagent.



## Theory of Protein BCA assay

The Protein BCA assay uses bicinchoninic acid (BCA) as the detection reagent for $Cu^{+1}$, which is formed when $Cu^{+2}$ is reduced by certain proteins in an alkaline environment. A purple reaction product is formed by the chelation of two molecules of BCA with one cuprous ion ($Cu^{+1}$). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm and baseline-corrected using the absorbance value at 750 nm. Pre-formulated kits of BCA reagent and $CuSO_4$ are available from us or a local distributor.

## Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop Ultra instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop Ultra pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

## Working with standard curves

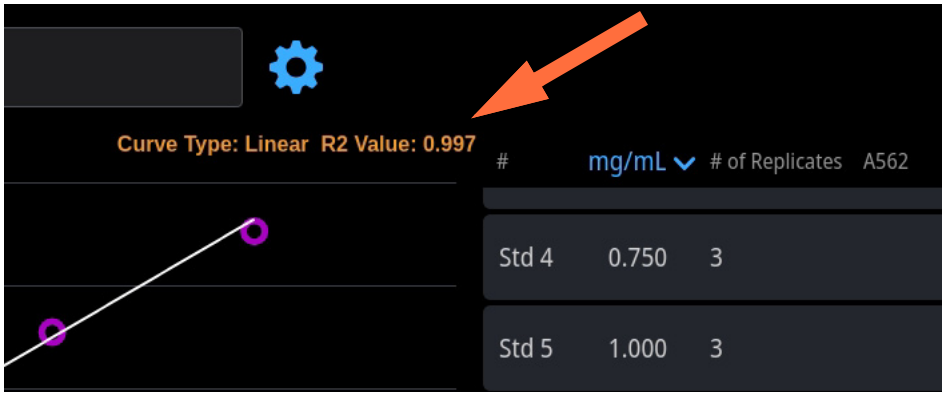
A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve or import standard from a previously run experiment.
- To import a previously run standard curve, from the application setup screen, select the **Load Standards** option, select one of the available previously run standard curves and select **Save**.
- Prepare standards and unknown samples the same way. See the kit manufacturer's guidelines and recommendations.
  - **All reference and standards solutions** should be the same buffer used to resuspend the samples plus the same volume of reagent added to the samples.
  - **First standard** is a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement. This application requires both.)
  - **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard.
- Use the **Setup Standards** option in the application setup screen to enter concentration values for the standards and to specify how standards and samples will be measured (number of replicates, curve type, etc.).
  - Depending on the Curve Type setting, a standard curve can be generated using two or more standards.
  - The software **requires one reference measurement** and allows **up to 7 standards**.
  - **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest.
- For all colorimetric assays except Protein Pierce 660, **blank the instrument** with DI $H_2O$ (deionized water). For Protein Pierce 660, blank with the reference solution (see below).
- **Measure the reference and all standards** before you start analyzing samples. After the first sample has been measured, no additional changes are allowed to the standard curve.

As you measure the standards, a measurement screen appears, similar to the measurement screens for samples.

When using the PC control software, select the **Curve** tab to see the standard curve as you build it.

The $R^2$ value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; all points lie exactly on the curve).




After the minimum number of standards has been measured for the selected curve type, a message similar to the following appears:


**Remeasure standards:** returns to standard measurement screen, where previously run standards can be selected and rerun.

– From standards measurement screen, standard curve screen, or standards data table, press and hold the row to show **Sample Details** box.

– Select **Remeasure** then select **Yes** to confirm.

**Load more standards**: returns to the setup screen where you can add or edit the concentration value for any standard and then select **Save** to measure. This option will be unavailable if you have already measured the maximum allowable amount of standards.

**Measure samples**: continues to sample measurement screen, after which standards can no longer be edited.

> **Note** You can add, edit or delete a standard any time before selecting **Measure Samples**.

**Add standard:**

– From standards measurement screen, select  .

– Select the next empty Concentration field and enter the concentration value for the new standard.

– Select **Save**.

**Edit standard:**

– From standards measurement screen, select  .

– Select the Concentration field and edit the concentration value.

– Select **Save**.

**Delete standard:**

– From standards measurement screen, standard curve screen, or standards data table, press and hold the row to show Standard Details box.

– Select  .

– Select **Yes** to confirm.

The standard no longer appears in the table on the measurement screen and its concentration value no longer appears on the setup screen.

> **Note** You can use this method to delete the reference measurement; however, a new reference must be measured immediately afterwards.

- After all standards have been measured for the selected curve type, the message "Standards Completed" will appear. If the message "Invalid Standards" appears after all entered standards have been measured, try:

  – selecting a different curve type

  – remeasuring standards using the correct standard material

> **Note** This message is only an indicator that the required minimum number of points has been established for the selected curve type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

# Measure Protein BCA

The Protein BCA assay uses bicinchoninic acid as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is useful for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm, which rules out direct protein measurements at 280 nm or 205 nm. This application measures absorbance at 562 nm and uses a standard curve to calculate protein concentration. A single-point baseline correction is applied.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

## Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein BCA**.
2. Configure any of the setup options if desired and select **Save**. (Specify a curve type and number of replicates for each standard and enter the concentration of each standard.)

   **Tip**: For this assay, we recommend setting **Curve Type** to “Linear”.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Measure blank:
   - Pipette 2 µL of DI $H_2O$ onto lower pedestal and lower arm, or insert DI $H_2O$ blanking cuvette into cuvette holder

     **Tip**: If using a cuvette, make sure to align cuvette light path with instrument light path.

- Select **Blank** and wait for measurement to complete.

  **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements).

- Lift arm and clean both pedestals with new laboratory wipe, or remove cuvette.

5. Measure reference standard:
   - Pipette 2 µL of reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details).
   - Select **Measure** and wait for measurement to complete.

     **Tip**: If Auto-Measure is On, the standard measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements).

   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
6. Measure remaining standards:
   - Pipette 2 µL of standard 1 onto pedestal, or insert standard 1 cuvette.
   - Select **Measure** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
   - Repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to remeasure standards, load more standards or begin measuring samples).
   - If finished measuring standards, select **Measure Samples** (Swipe left to view standard curve) when using the local control software or select the Curve tab when using the PC control software.
7. Measure samples:
   - Pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette.
   - Select **Measure Samples** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.

8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

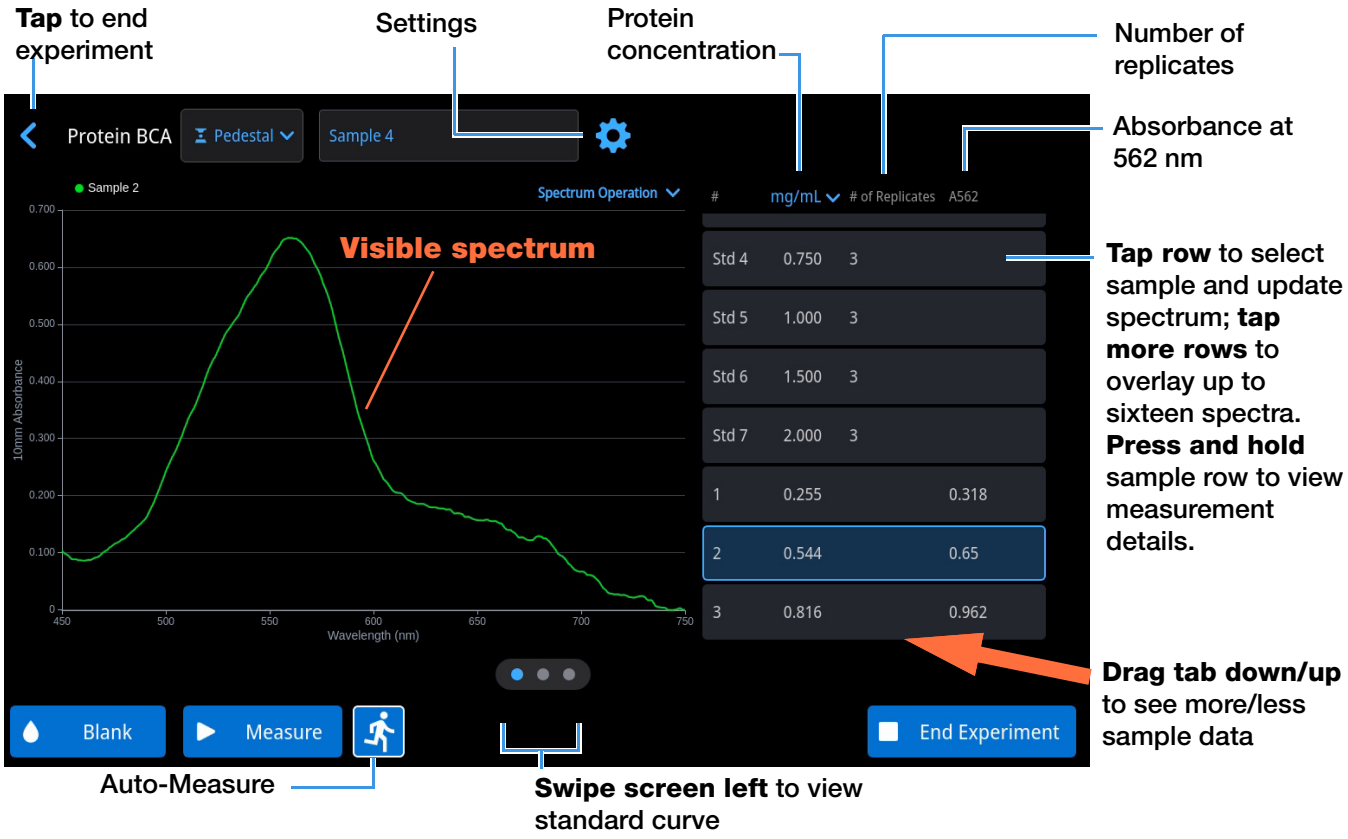
## Related Topics

- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Protein BCA measurement screen (local control software)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. When using the NanoDrop Ultra local control software, the standard curve is available by swiping left from the measurement screen (or in History).




**Note**

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

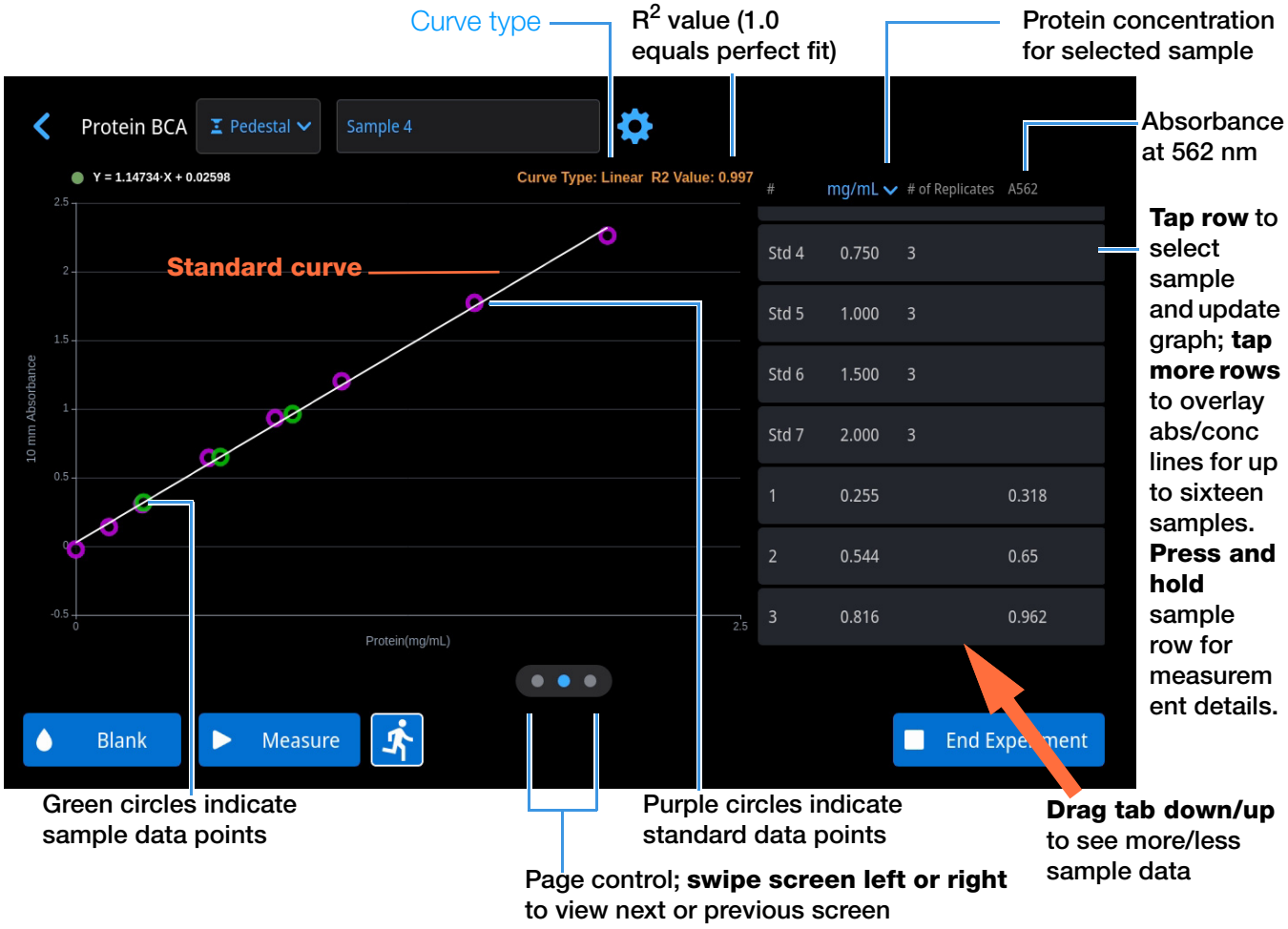
## Protein BCA measurement screen (PC control software)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Protein BCA standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

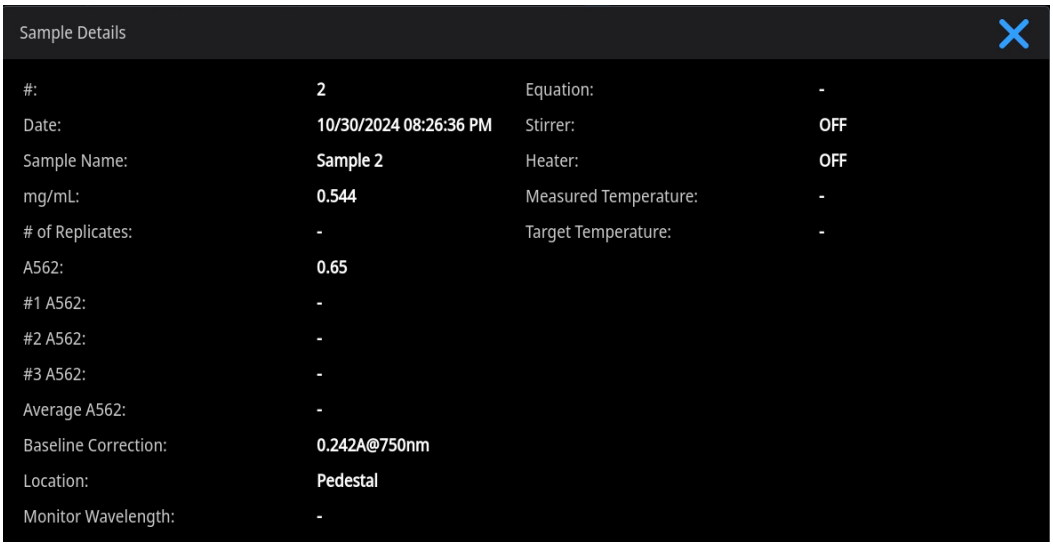
The $R^2$ value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).




When using the NanoDrop Ultra PC control software, select **Curve** to view the Standard curve.

## Reported values

The initial screen that appears after each measurement and the standards screen (see previous image) show a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

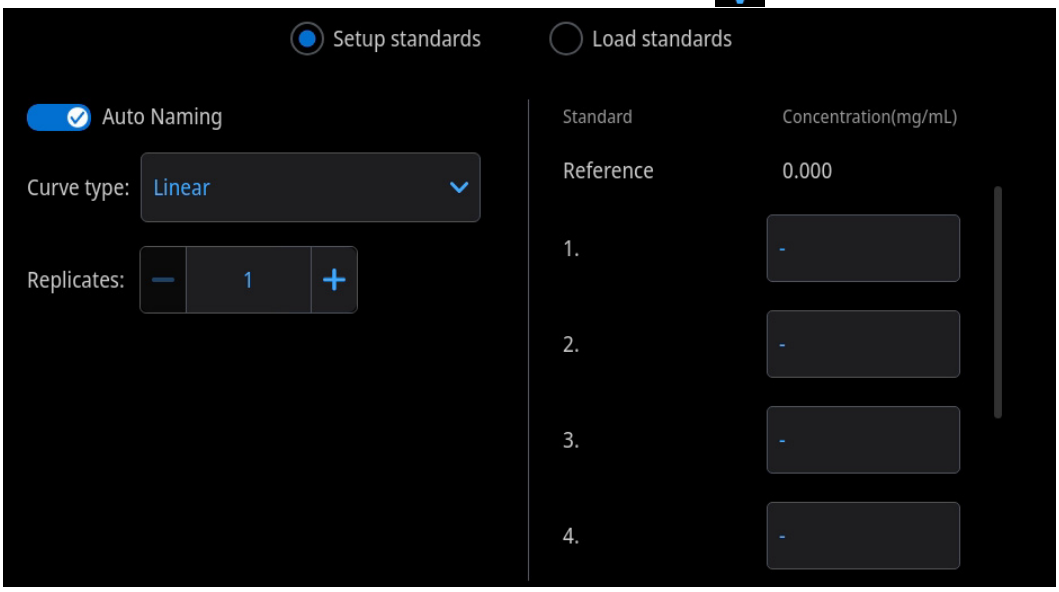



### Related Topics

- Example standard curve
- Basic Instrument Operations
- Protein A280 Calculations

# Settings

The Protein BCA Setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein BCA settings, from the Protein BCA measurement screen, select the setup icon .




**Note** You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After selecting **Measure Samples**, these settings cannot be changed.

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | "On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

| Setting | Available Options | Description |
|---|---|---|
| Curve Type | | Specify type of equation used to create standard curve from standard concentration values.<br><br>Available options:<br>– **Linear**: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard)<br>– **Interpolation**: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard)<br>– **2nd order polynomial**: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards)<br>– **3rd order polynomial**: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards) |
| Replicates | | Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value.<br><br>**Note**: Replicates setting cannot be changed after the first standard has been measured. |
| Standards | | Enter actual concentration value of each standard.<br><br>**Note**: Concentration values can be entered in any order but the standards must be measured in the order they were entered.<br><br>If you also want to use previously measured standards, select **Load Standards**. |

# Protein Bradford

Measures total protein concentration of unpurified protein samples using a Coomassie Blue dye colorimetric detection reagent.

Measure Protein Bradford

Reported Results

Settings

Detection Limits

## Theory of Protein Bradford assay

The Protein Bradford assay uses the protein-induced absorbance shift of Coomassie Blue dye to determine total protein concentration. The bound protein-dye complex is measured at 595 nm and baseline-corrected using the absorbance value at 750 nm. Pre-formulated kits of stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant are available from us or a local distributor.

To maximize reliability with the Protein Bradford assay:

- **Work quickly and do not allow prepared standards or samples to sit longer than necessary**. Coomassie dye-dye and Coomassie dye-protein aggregates can form particulates with increasing development time, resulting in significant fluctuations in absorbance readings.
- **Measure standards and samples in triplicate** using a new aliquot for each measurement. For pedestal measurements, the total analyte (protein-dye) signal at 595 nm is limited to ~0-0.150A due to the pedestal's 1.0 mm pathlength, the Coomassie dye concentration, and the acidic pH.

**Note** If you have a NanoDrop Ultra$^{C}$ or a NanoDrop Ultra$^{C}$ FL model instrument, using the cuvette option will result in a higher absorbance signal.

## Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop Ultra instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop Ultra pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

### Protein Bradford standard curves

A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve by selecting the **Setup standards** option or import standard from a previously run experiment by selecting **Load standards** on the application setup screen.

For more information, see Working with Standard Curves.

## Measure Protein Bradford

The Protein Bradford assay uses Coomassie Blue dye as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is useful for measuring dilute protein solutions that require lower detection sensitivity or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm, which rules out direct protein measurements at 280 nm or 205 nm. This application measures absorbance at 595 nm and uses a standard curve to calculate protein concentration. See Working with Standard Curves for more information. A single-point baseline correction is applied.

#### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein Bradford**.
2. Configure any of the setup options if desired and select **Save**. (Specify a curve type and number of replicates for each standard and enter the concentration of each standard.)

   **Tip**: For this assay, set **Curve Type** to “2nd Order Polynomial” and **Replicates** to 3.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Measure blank:
   - Pipette 2 µL DI $H_2O$ of onto lower pedestal and lower arm, or insert DI $H_2O$ blanking cuvette into cuvette holder.

     **Tip**: If using a cuvette, make sure to align cuvette light path with instrument light path.
   - Select **Blank** and wait for measurement to complete.

     **Tip**: If Auto-Measure is On, the standard measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements).
   - Lift arm and clean both pedestals with new laboratory wipe, or remove cuvette.
5. Measure reference standard:
   - Pipette 2 µL of reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details).
   - Select **Measure** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
6. Measure remaining standards:
   - Pipette 2 µL of standard 1 onto pedestal, or insert standard 1 cuvette.
   - Select **Measure** and wait for measurement to complete.

- Lift arm and clean both pedestals with new wipe, or remove cuvette.
- If Replicates setting is greater than 1, repeat measurement.
- Repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to remeasure standards, load more standards, or begin measuring samples).
- If finished measuring standards, select **Measure Samples** (Swipe left to view standard curve) when using the local control software or select the Curve tab when using the PC control software.

7. Measure samples:
   - Pipette 2 µL of sample 1 onto pedestal, or insert sample 1 cuvette.
   - Select **Measure samples** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

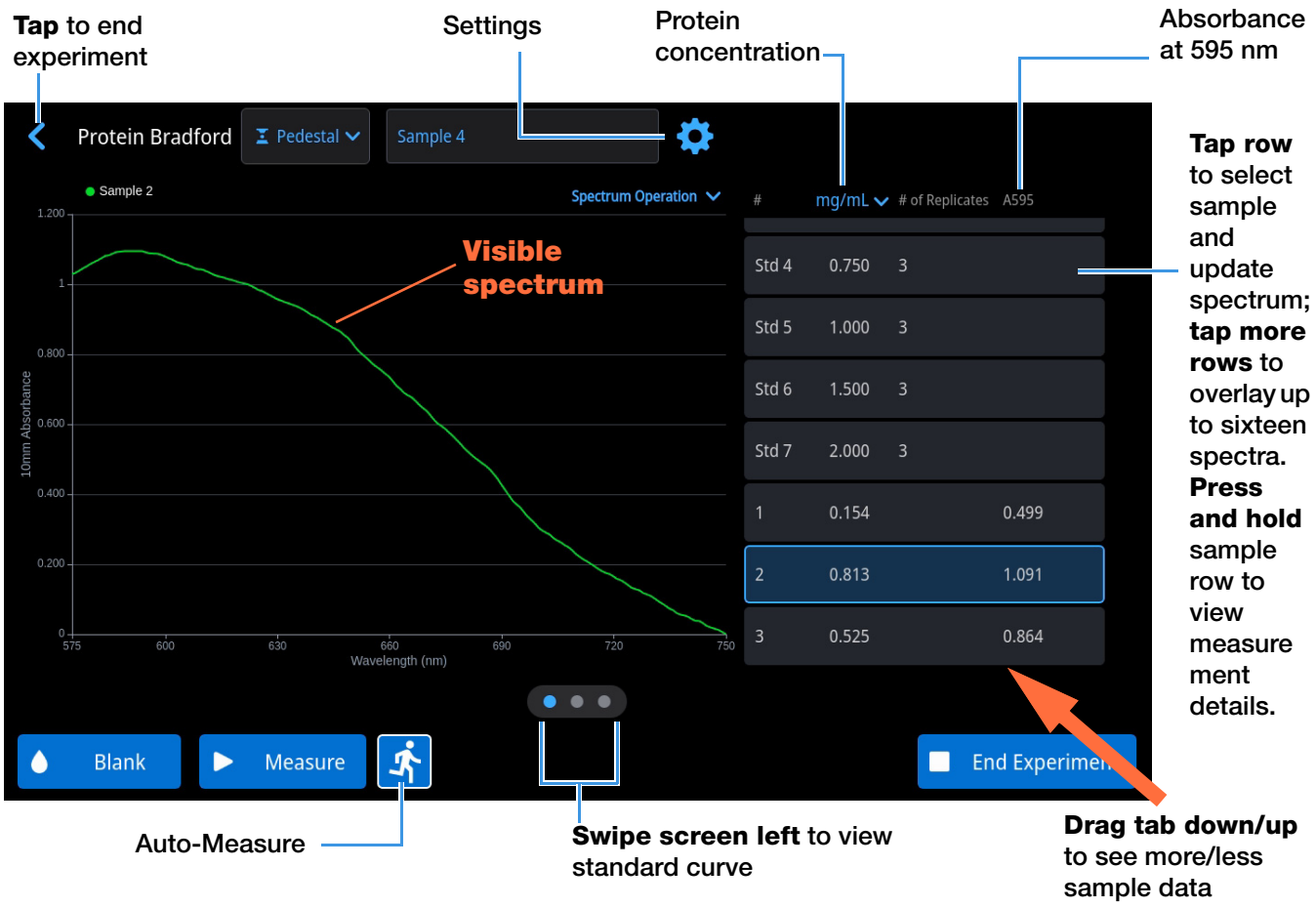
## Related Topics

- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Protein Bradford measurement screen (local control software)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. When using the NanoDrop Ultra local control software, the standard curve is also available by swiping left from the measurement screen (or in History).




**Note**

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

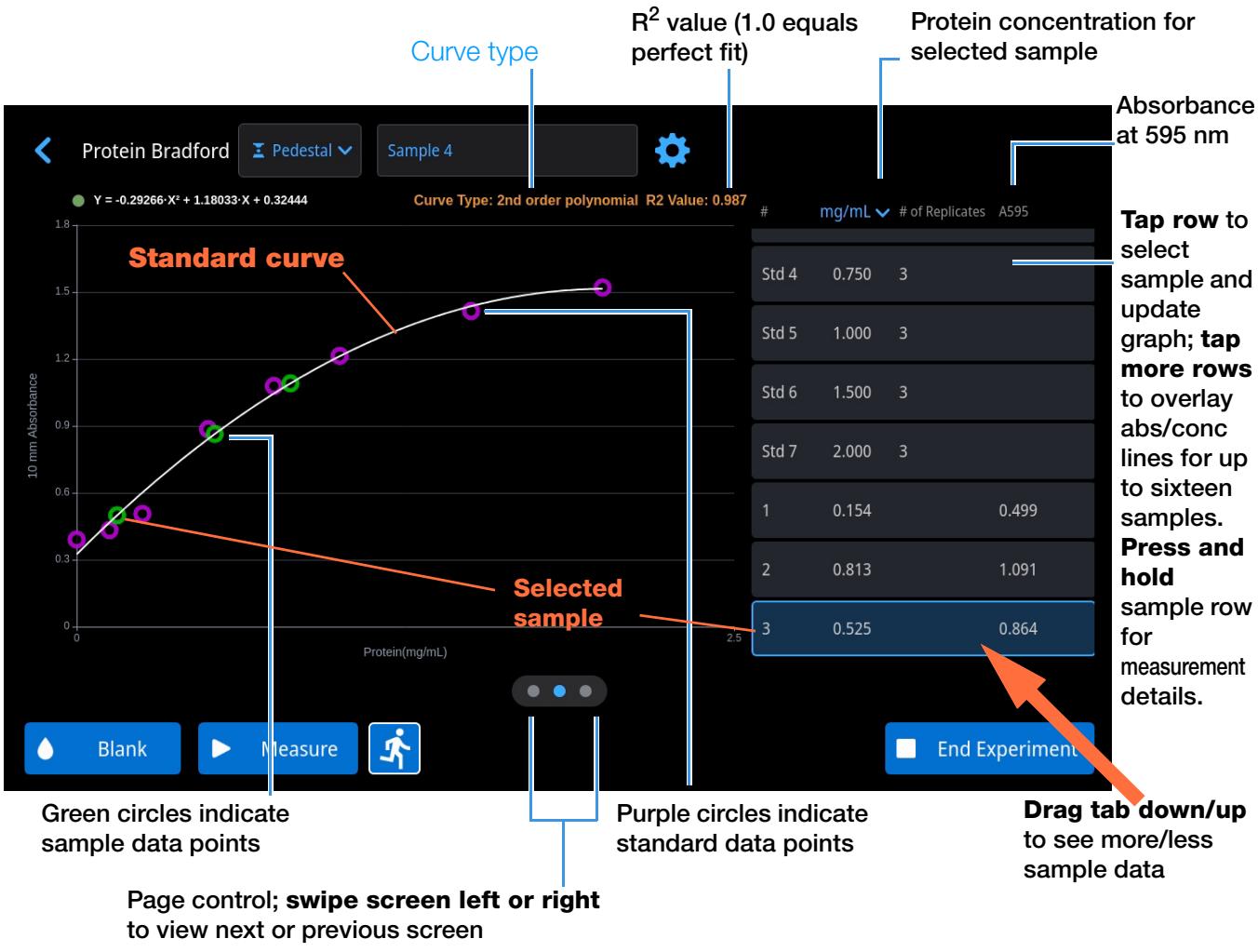
## Protein Bradford measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Protein Bradford standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

The $R^2$ value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



When using the NanoDrop Ultra PC control software, select **Curve** to view the Standard curve.

**Note**

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Reported values

The initial screen that appears after each measurement and the standards screen (see previous image) show a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

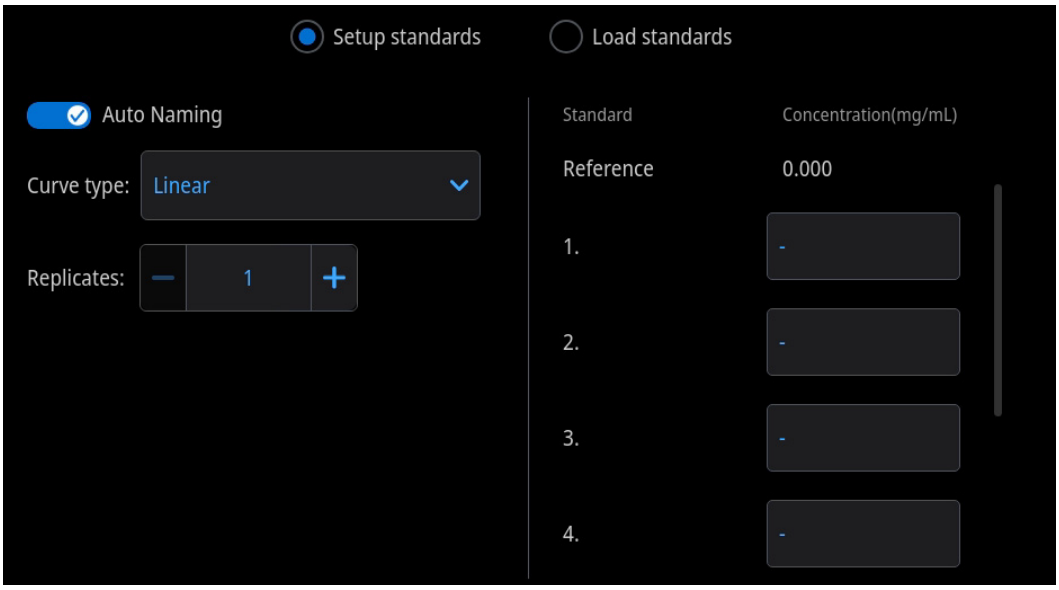
| | | | |
|---|---|---|---|
| #: | 3 | Equation: | - |
| Date: | 10/30/2024 03:15:02 PM | Stirrer: | OFF |
| Sample Name: | Sample 3 | Heater: | OFF |
| mg/mL: | 0.525 | Measured Temperature: | - |
| # of Replicates: | - | Target Temperature: | - |
| A595: | 0.864 | | |
| #1 A595: | - | | |
| #2 A595: | - | | |
| #3 A595: | - | | |
| Average A595: | - | | |
| Baseline Correction: | 0.243A@750nm | | |
| Location: | Pedestal | | |
| Monitor Wavelength: | - | | |

## Related Topics

- Example standard curve
- Basic Instrument Operations
- Protein A280 Calculations

# Settings

The Protein Bradford Setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein Bradford settings from the Protein Bradford measurement screen, select the setup icon .




**Note** You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After selecting **Measure Samples**, these settings cannot be changed.

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | "On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

| Setting | Available Options | Description |
|---|---|---|
| Curve Type | | Specify type of equation used to create standard curve from standard concentration values. Available options:<br>– **Linear**: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard)<br>– **Interpolation**: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard)<br>– **2nd order polynomial**: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards)<br>– **3rd order polynomial**: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards) |
| Replicates | | Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value.<br>**Note**: Replicates setting cannot be changed after the first standard has been measured. |
| Standards | | Enter actual concentration value of each standard.<br>**Note**: Concentration values can be entered in any order but the standards must be measured in the order they were entered.<br>If you also want to use previously measured standards, select **Load Standards**. |

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# Protein Lowry

Measures total protein concentration of unpurified protein samples using a Folin-Ciocalteu colorimetric detection reagent.

Measure Protein Lowry

Reported Results

Settings

Detection Limits

## Theory of Protein Lowry assay

The Protein Lowry assay involves the reaction of protein with cupric sulfate in alkaline solution, resulting in the formation of tetradentate copper-protein complexes. The Folin-Ciocalteu reagent is effectively reduced in proportion to the chelated copper-complexes. The water-soluble blue reaction product is measured at 650 nm and baseline-corrected using the absorbance value at 405 nm. Pre-formulated kits of Folin-Ciocalteu reagent and $CuSO_4$ are available from us or a local distributor.

## Protein assay kits and protocols

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

## Protein Lowry standard curves

A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve by selecting the **Setup standards** option or import standard from a previously run experiment by selecting **Load Standards** on the application setup screen.

For more information, see Working with Standard Curves.

# Measure Protein Lowry

The Protein Lowry assay uses Folin-Ciocalteu as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is an alternative to the other colorimetric applications for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm. This application measures absorbance at 650 nm and uses a standard curve to calculate protein concentration. See Working with Standard Curves for more information. A single-point baseline correction is applied.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein Lowry**.
2. Configure any of the setup options if desired and select **Save**.

   **Tip**: For this assay, we recommend setting **Curve Type** to "2nd Order Polynomial."
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Measure blank:
   - Pipette 2 µL DI $H_2O$ onto lower pedestal and lower arm, or insert DI $H_2O$ blanking cuvette into cuvette holder.

     **Tip**: If using a cuvette, make sure to align cuvette light path with instrument light path.

- Select **Blank** and wait for measurement to complete.

  **Tip**: If Auto-Measure is On, the standard measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements).

- Lift arm and clean both pedestals with new laboratory wipe, or remove cuvette.

5. Measure reference standard:
   - Pipette 2 µL of reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details).
   - Select **Measure** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
6. Measure remaining standards:
   - Pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette.
   - Select **Measure** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
   - Repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to remeasure standards, load more standards, or begin measuring samples).
   - If finished measuring standards, select **Measure Samples**. (Swipe left to view standard curve) when using the local control software or select the Curve tab when using the PC control software.
7. Measure samples:
   - Pipette 2 µL ofsample 1 onto pedestal, or insert sample 1 cuvette.
   - Select **Measure Samples** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

## Related Topics

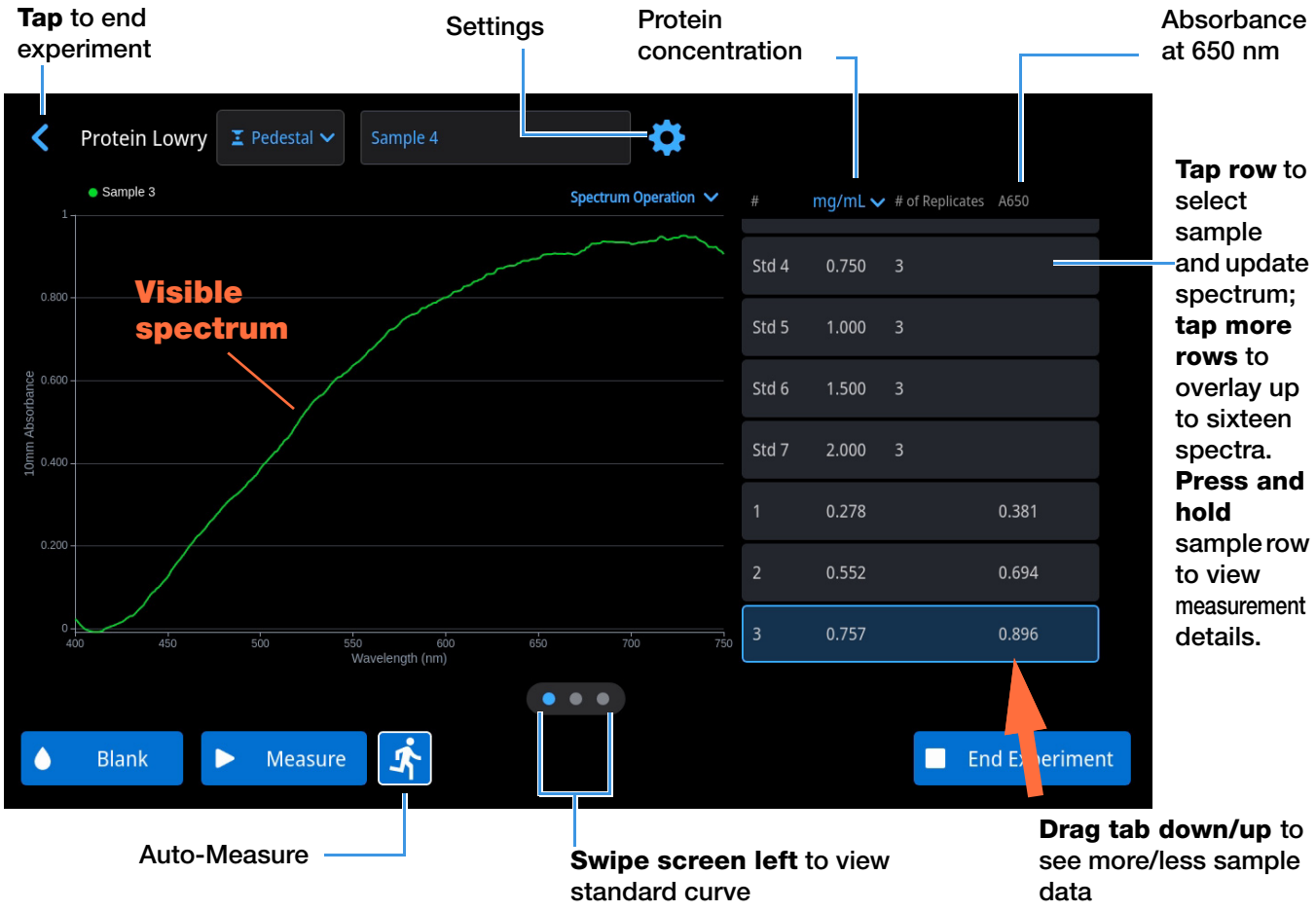
- Working with standard curves

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Protein Lowry measurement screen (local control software)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. When using the NanoDrop Ultra local control software, the standard curve is available by swiping left from the measurement screen (or in History).




**Note**

- A baseline correction is performed at 405 nm (absorbance value at 405 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

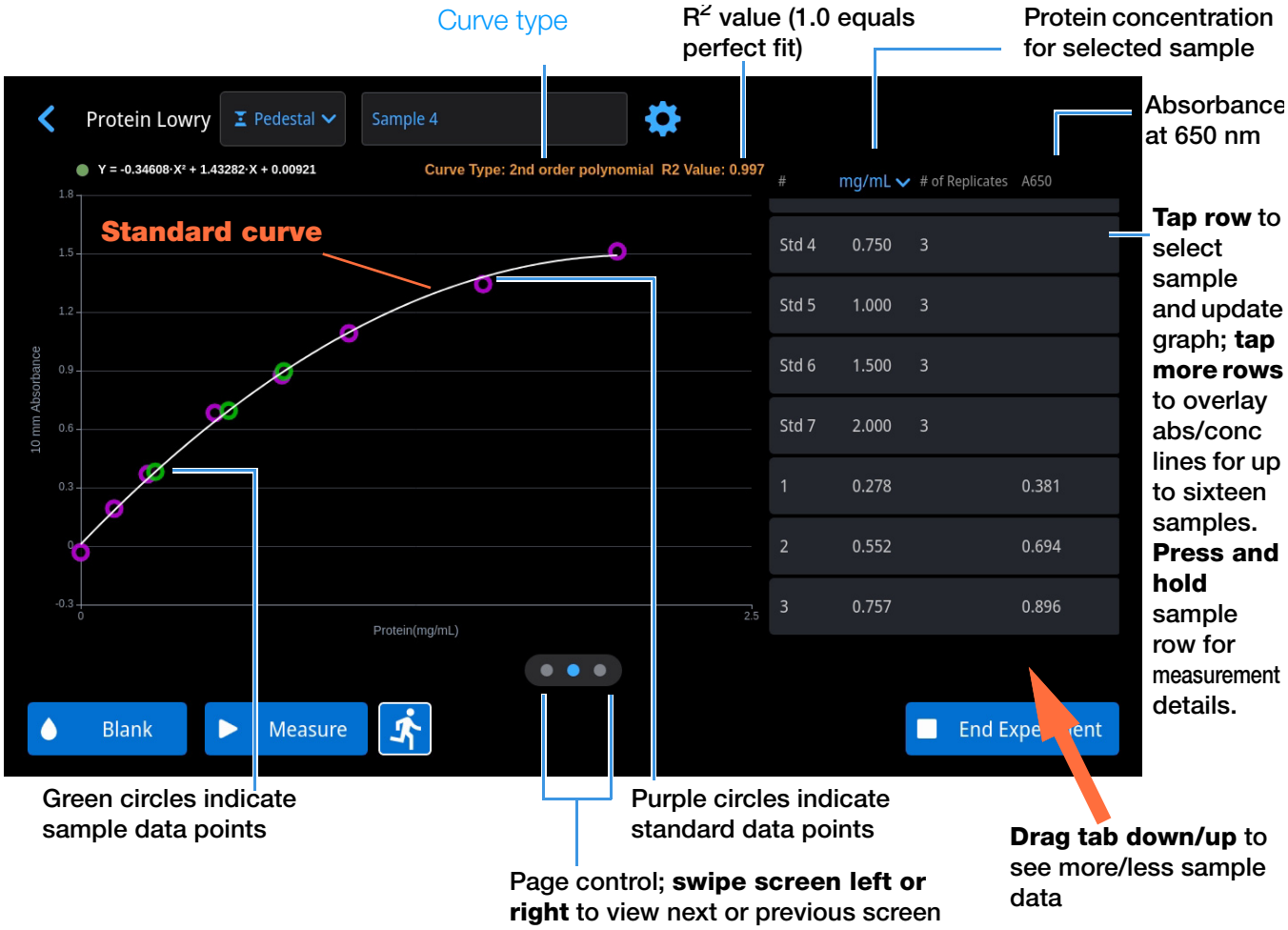
## Protein Lowry measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Protein Lowry standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

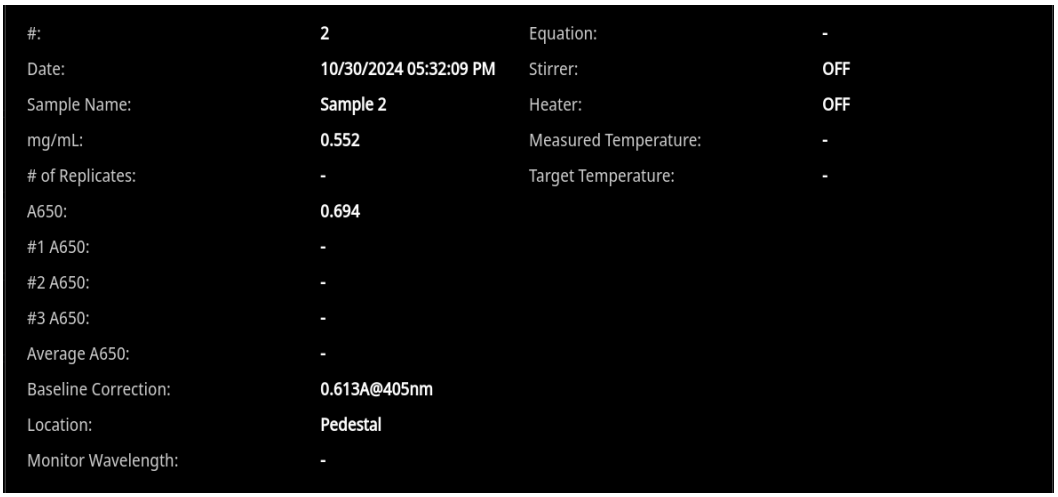
The $R^2$ value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).




When using the NanoDrop Ultra PC control software, select **Curve** to view the Standard curve.

## Reported values

The initial screen that appears after each measurement and the standards screen (see previous image) show a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



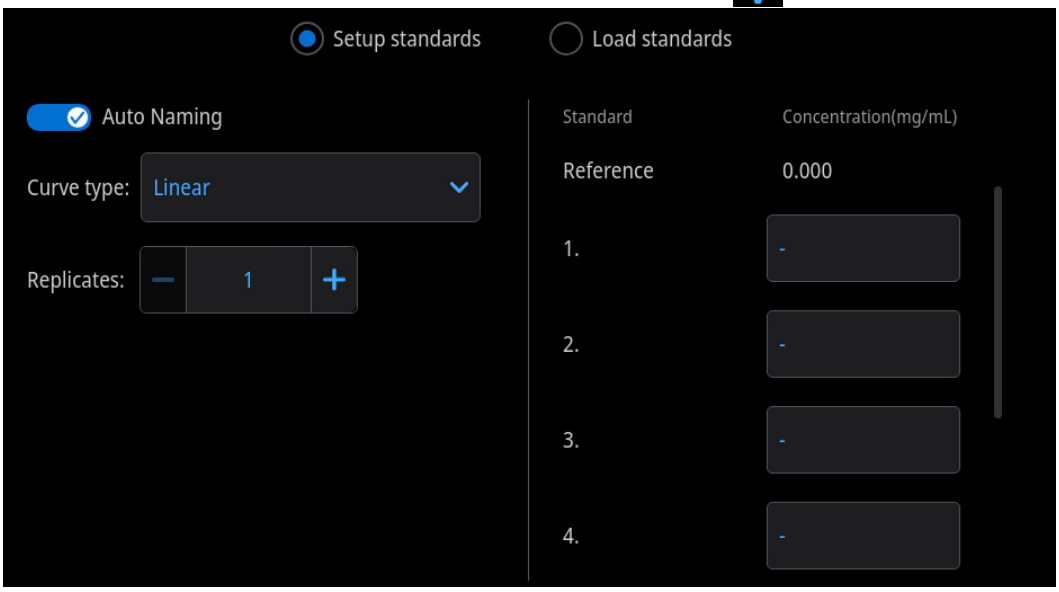
| | | | |
|---|---|---|---|
| #: | 2 | Equation: | - |
| Date: | 10/30/2024 05:32:09 PM | Stirrer: | OFF |
| Sample Name: | Sample 2 | Heater: | OFF |
| mg/mL: | 0.552 | Measured Temperature: | - |
| # of Replicates: | - | Target Temperature: | - |
| A650: | 0.694 | | |
| #1 A650: | - | | |
| #2 A650: | - | | |
| #3 A650: | - | | |
| Average A650: | - | | |
| Baseline Correction: | 0.613A@405nm | | |
| Location: | Pedestal | | |
| Monitor Wavelength: | - | | |

## Related Topics

- Example standard curve
- Basic Instrument Operations
- Protein A280 Calculations

# Settings

The Protein Lowry setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein Lowry settings, from the Protein Lowry measurement screen, select the setup icon .




**Note** You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After selecting **Measure Samples**, these settings cannot be changed.

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | "On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

| Setting | Available Options | Description |
|---|---|---|
| Curve Type | | Specify type of equation used to create standard curve from standard concentration values. Available options:<br>– **Linear**: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard)<br>– **Interpolation**: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard)<br>– **2nd order polynomial**: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards)<br>– **3rd order polynomial**: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards) |
| Replicates | | Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value.<br><br>**Note**: Replicates setting cannot be changed after the first standard has been measured. |
| Standards | | Enter actual concentration value of each standard.<br><br>**Note**: Concentration values can be entered in any order but the standards must be measured in the order they were entered.<br><br>If you also want to use previously measured standards,select **Load Standards**. |

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# Protein Pierce 660

Measures total protein concentration of unpurified protein samples using a proprietary colorimetric detection reagent.

Measure Protein Pierce 660

Reported Results

Settings

Detection Limits

## Theory of Protein Pierce 660 assay

The Protein Pierce 660 assay is based on the binding of a proprietary dye-metal complex to protein in acidic conditions that causes a shift in the dye's absorption maximum, which is measured at 660 nm. The dye-metal complex is reddish-brown and changes to green upon protein binding. The color change is produced by deprotonation of the dye at low pH facilitated by interactions with positively charged amino acid groups in proteins. The dye interacts mainly with basic residues in proteins such as histidine, arginine and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine. The reaction product is measured at 660 nm and baseline-corrected using the absorbance value at 750 nm.

The color produced in the assay is stable and increases in proportion to a broad range of increasing protein concentrations. An optional Ionic Detergent Compatibility Reagent (IDCR) may be added to the assay reagent to increase compatibility with high amounts of ionic detergents, including Laemmli SDS sample buffer with bromophenol blue. The IDCR dissolves completely by thorough mixing and has no effect on the assay. Pre-formulated kits of the protein binding material are available from us or a local distributor. For information about IDCR, refer to the kit manufacturer.

## Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop Ultra instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop Ultra pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

### Protein Pierce standard curves

A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve by selecting the **Setup standards** option or import standard from a previously run experiment by selecting **Load Standards** on the application setup screen.

For more information, see Working with Standard Curves.

## Measure Protein Pierce 660

The Protein Pierce 660 assay uses a proprietary protein binding material as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is suitable for protein solutions that contain high concentrations of detergents, reducing agents and other commonly used reagents. The Pierce 660 application measures absorbance at 660 nm and uses a standard curve to calculate protein concentration (see Working with Standard Curves for more information). A single-point baseline correction is applied.

#### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **Proteins** tab, then select **Protein Pierce 660**.
2. Configure any of the setup options if desired and select **Save**. (Specify a curve type and number of replicates for each standard and enter the concentration of each standard.)

   **Tip**: For this assay, we recommend setting **Curve Type** to “Linear”.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Measure blank:
   - Pipette 2 µL of reference solution onto lower pedestal and lower arm, or insert reference solution blanking cuvette into cuvette holder (reference solution should contain none of the standard protein stock; see Working With Standard Curves for details).

     **Tip**: If using a cuvette, make sure to align cuvette light path with instrument light path.
   - Select **Blank** and wait for measurement to complete.

     **Tip**: If Auto-Measure is On, the standard measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements).
   - Lift arm and clean both pedestals with new laboratory wipe, or remove cuvette.
5. Measure reference standard:
   - Pipette 2 µL ofreference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details).
   - Select **Measure** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
6. Measure remaining standards:
   - Pipette 2 µL of standard 1 onto pedestal, or insert standard 1 cuvette.

- Select **Measure** and wait for measurement to complete.
- Lift arm and clean both pedestals with new wipe, or remove cuvette.
- If Replicates setting is greater than 1, repeat measurement.
- Repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to remeasure standards, load more standards, or begin measuring samples).
- If finished measuring standards, select **Measure Samples** (swipe left to view standard curve) when using the local control software or select the Curve tab when using the PC control software.

7. Measure samples:
   - Pipette 2 µL of sample 1 onto pedestal, or insert sample 1 cuvette.
   - Select **Measure Samples** and wait for measurement to complete.
   - Lift arm and clean both pedestals with new wipe, or remove cuvette.
   - If Replicates setting is greater than 1, repeat measurement.
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

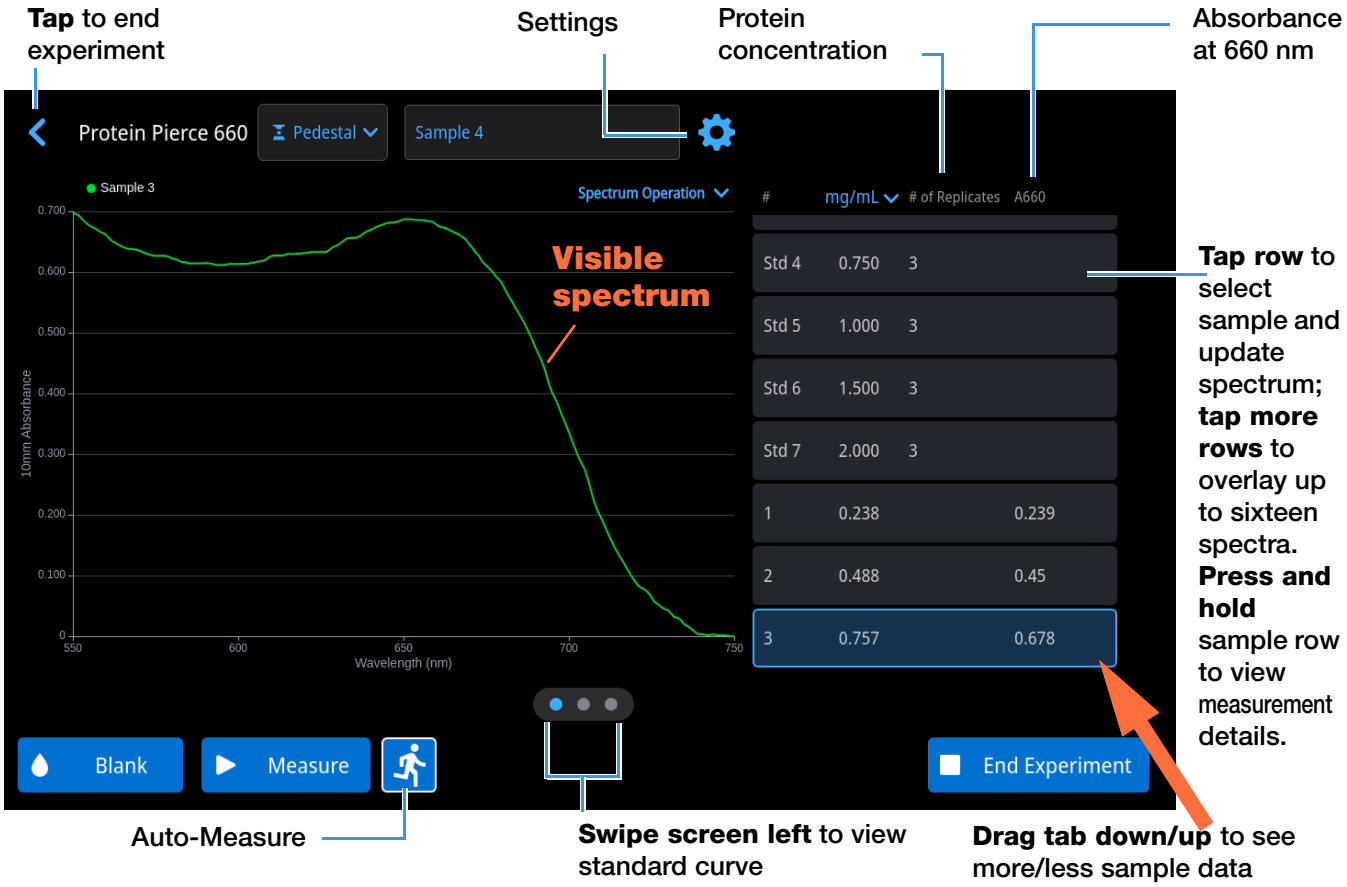
## Related Topics

- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Protein Pierce 660 measurement screen (local control software)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. In the NanoDrop Ultra local control software, the standard curve is also available by swiping left from the measurement screen (or in History).




**Note**

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

When using the NanoDrop Ultra PC control software, select **Curve** to view the Standard curve.

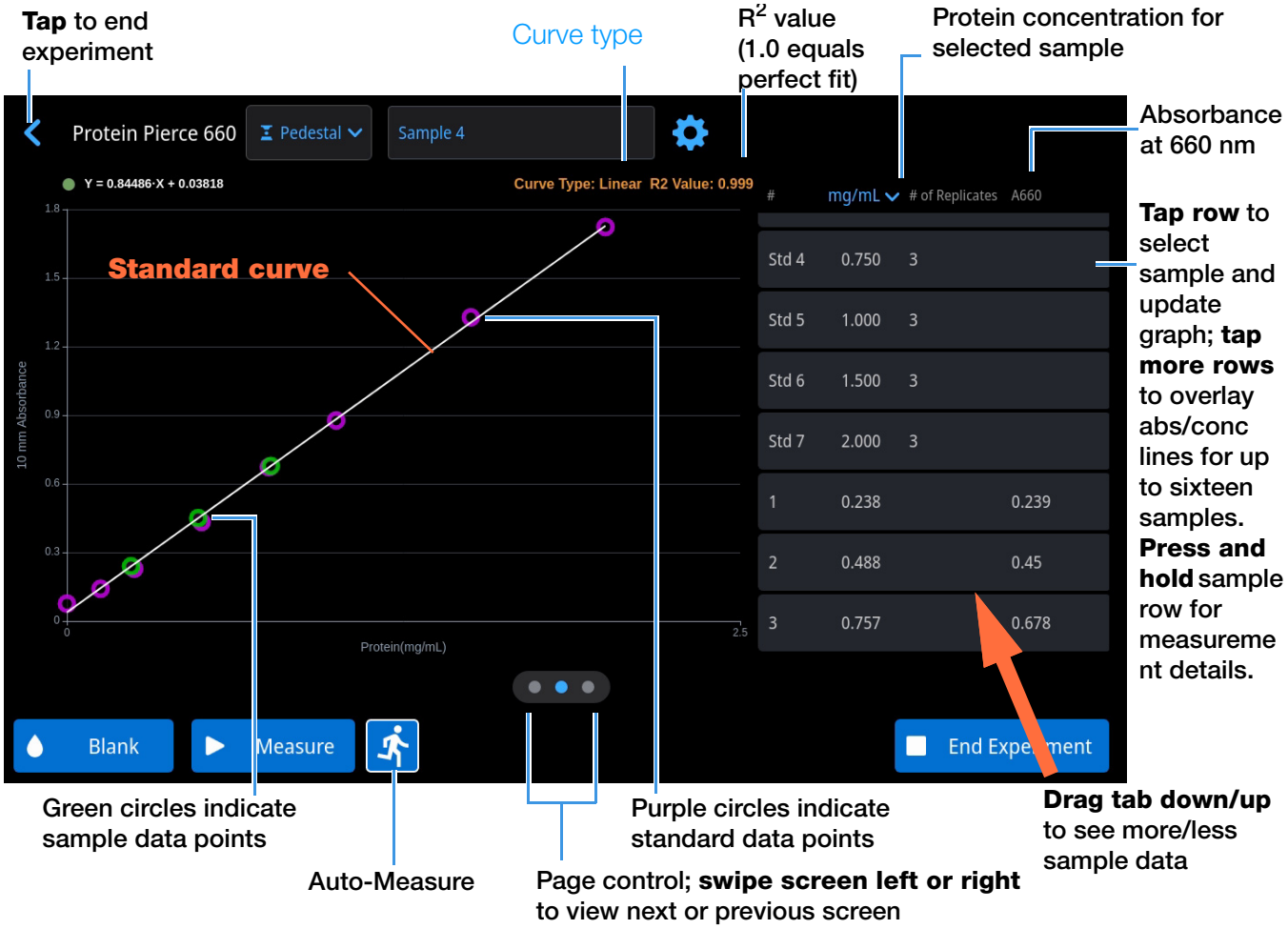
## Protein Pierce 660 measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Protein Pierce 660 standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

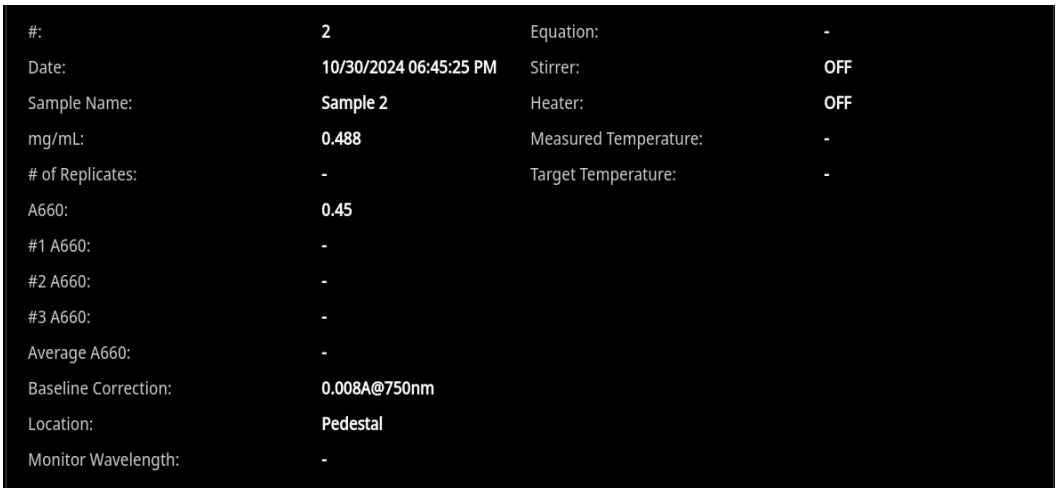
The $R^2$ value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).




When using the NanoDrop Ultra PC control software, select **Curve** to view the Standard curve.

## Reported values

The initial screen that appears after each measurement and the standards screen (see previous image) show a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



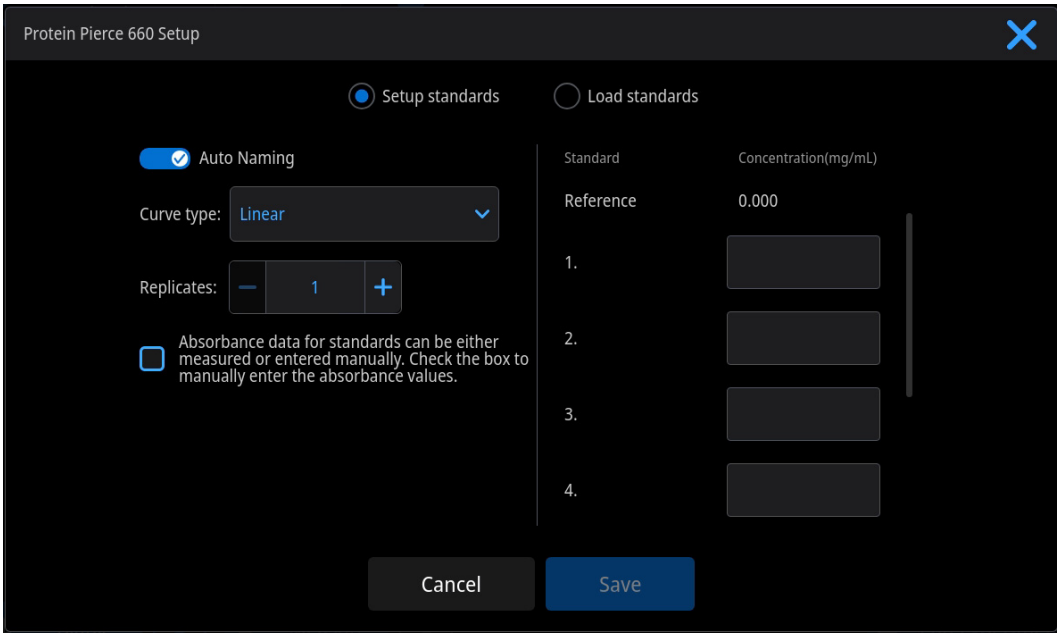
| | | | |
|---|---|---|---|
| #: | 2 | Equation: | - |
| Date: | 10/30/2024 06:45:25 PM | Stirrer: | OFF |
| Sample Name: | Sample 2 | Heater: | OFF |
| mg/mL: | 0.488 | Measured Temperature: | - |
| # of Replicates: | - | Target Temperature: | - |
| A660: | 0.45 | | |
| #1 A660: | - | | |
| #2 A660: | - | | |
| #3 A660: | - | | |
| Average A660: | - | | |
| Baseline Correction: | 0.008A@750nm | | |
| Location: | Pedestal | | |
| Monitor Wavelength: | - | | |

## Related Topics

- Example standard curve
- Basic Instrument Operations
- Protein A280 Calculations

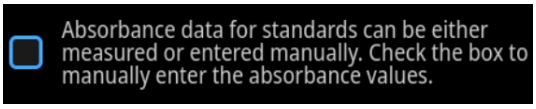
# Settings

The Protein Pierce 660 setup screen appears after you select the application from the Proteins tab on the home screen. To show the Protein Pierce 660 settings, from the Protein Pierce 660 measurement screen, select the setup icon .




**Note** You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After selecting **Measure Samples**, these settings cannot be changed.

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | "On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

| Setting | Available Options | Description |
|---|---|---|
| Curve Type | | Specify type of equation used to create standard curve from standard concentration values. Available options:<br>– **Linear**: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard)<br>– **Interpolation**: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard)<br>– **2nd order polynomial**: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards)<br>– **3rd order polynomial**: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards) |
| Replicates | | Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value.<br>**Note**: Replicates setting cannot be changed after the first standard has been measured. |
| Standards | | Enter actual concentration value of each standard.<br>**Note**: Concentration values can be entered in any order but the standards must be measured in the order in which they were entered.<br>If you also want to use previously measured standards, select **Load Standards**.<br>If you also want to enter previously measured absorbance values for the standards, select this check box:<br>  <br>and the enter absorbance values for all the standards. |

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6

# Fluorescence Applications

## dsDNA Fluorescence or RNA Fluorescence

Measure the concentration of double-stranded DNA (dsDNA) or RNA tagged with a fluorophore using fluorescence. These applications are designed for use with a NanoDrop Ultra Fluorescence Assay. These applications are only available for use on the NanoDrop Ultra FL and NanoDrop Ultra$^{C}$ FL and are not compatible with the standard cuvette mode.


Measure dsDNA Fluorescence or RNA Fluorescence

Reported Results

Settings

Detection Limits

Calculations

### Best practices for fluorescence measurements

- For untested contaminating substances, and for highest accuracy, the standards should be assayed under the same conditions as the experimental samples.
- Isolate and purify nucleic acid samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA, free nucleotides, proteins, some buffer components and dyes. See Preparing Samples for more information.
- Ensure the sample concentration is within the selected assay's limits.

- The NanoDrop Ultra Fluorescence Assays deliver optimal performance when all solutions are at room temperature (18-28 °C). Temperature fluctuations can influence the accuracy of the assays.
- Ensure pedestal surfaces are properly cleaned and conditioned.
- Vortex and centrifuge before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
- Follow best practices for micro-volume measurements.
- Use a 2 µL sample volume. See Recommended Sample Volumes for more information.
- During each experiment, you have the option to run a new standard curve or use values from a previous standard curve. To minimize variables that can affect performance, performing a new calibration for every new assay run is strongly recommended.
- No data are currently available that address the mutagenicity or toxicity of the **NanoDrop™ Ultra dsDNA BR Fluorescence Reagents** (the dye in Component A). These reagents are known to bind to nucleic acids. Treat buffers with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.
- The **NanoDrop™ Ultra RNA HS Fluorescence Assay** includes a high-quality rRNA standard. It is crucial to maintain the integrity and concentration of these standards for optimal performance. Therefore, we highly recommend treating the rRNA standards with the same care as any other RNA. This includes:
  - Using appropriate RNAse-free handling techniques, such as RNAse-free gloves, pipette tips, and tubes.
  - It is important to keep the tube lids closed whenever possible.
  - Avoid touching the pipette to the inside wall of the tube when withdrawing a sample.
  - After use, it is advisable to promptly return the rRNA standard to the refrigerator.

### Related Topics

- Measure a Micro-Volume Sample
- Best Practices for Micro-Volume Measurements
- Basic Instrument Operations

## Working with standard curves

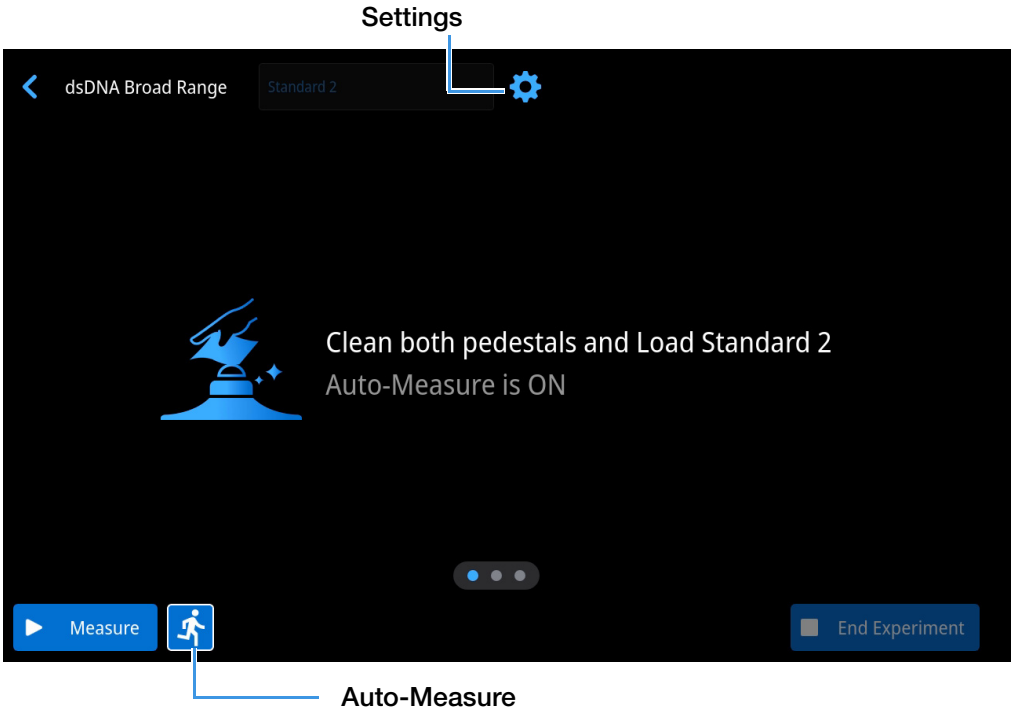
A standard curve is required for fluorescence analysis.

- Each experiment requires a standard curve. You can run a new standard curve or import standards from a previously run experiment.
- To import a previously run standard curve, select the **Load standards** option within the application setup screen, highlight a previously run standard curve, and select **Save**.
- To generate a new standard curve, select the **Setup standards** option within the application setup screen. Make any necessary changes to the application specific settings and then select **Save**.

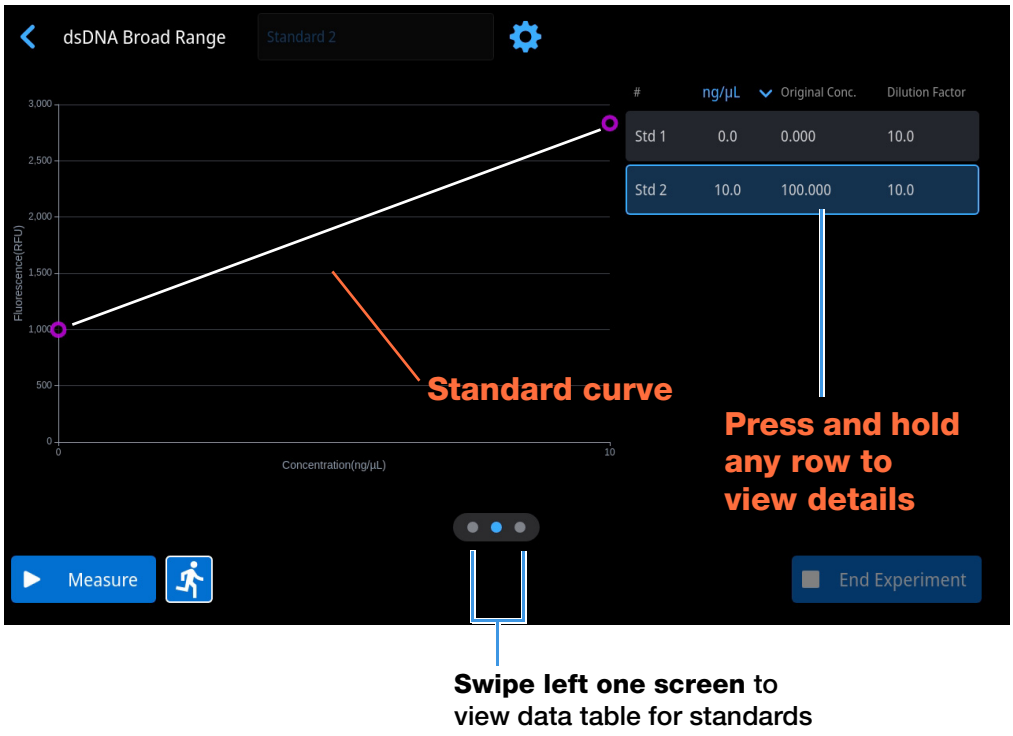
**Note** NanoDrop Ultra BR and HS Fluorescence Assays require 2 standards.

- **Measure all standards** before you start analyzing samples. After the first sample has been measured, no additional changes are allowed to the standard curve.

  As you measure the standards, a dialog screen appears, prompting the user to measure the remaining standards.



**Swipe left one screen** to see the standard curve as you build it. Here is an example:

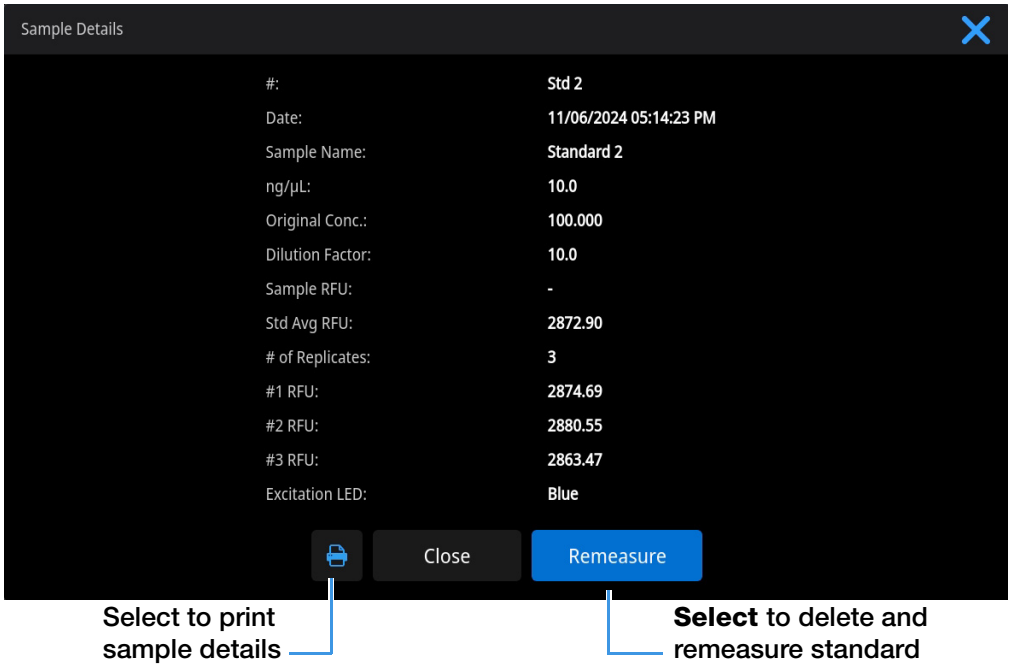



**Swipe left one screen** to view data table for standards

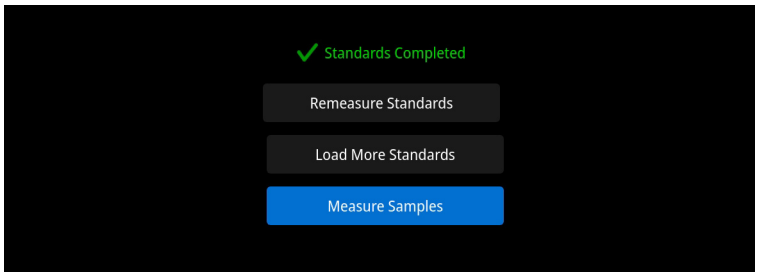
When using the Fluorometer application, an $R^2$ value will appear and indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; all points lie exactly on the curve).

**Swipe left one screen** to see the data table for the standards. Here is an example:


**Press and hold** a row in any of the previous screens to view details about an individual standard. Here is an example:



After the both standards have been measured for the selected curve type, a message similar to the following appears:




**Remeasure standards:** returns to standard measurement screen, where previously run standards can be selected and rerun.

– From the standard curve screen or standards data table, press and hold the row of the standard that is to be remeasured to show the **Sample Details** box.

– Select **Remeasure**.

**Load more standards**: returns to the setup screen where you can add additional standards to measure. A total of 1 reference and 7 standards can be used. This option will not appear when using the dsDNA Fluorescence or RNA Fluorescence applications as 2 standards must be used in those applications.

**Measure samples**: continues to sample measurement screen.

**Note** After both standards have been measured, the message "Standards Completed" should appear. If the "Invalid Standards" message appears after all standards have been measured, try remeasuring standards using the correct standard material.

# Measure dsDNA Fluorescence or RNA Fluorescence

Use the dsDNA Fluorescence and RNA Fluorescence applications paired with NanoDrop Ultra Fluorescence Assays to quantify dsDNA or RNA samples. These applications report nucleic acid concentration. The NanoDrop Ultra dsDNA Broad Range (BR), dsDNA High Sensitivity (HS), and RNA High Sensitivity (HS) Fluorescence Assays contain selective dyes that fluoresce only when bound to the target molecule.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals. See Working with standard curves for more information on the options available when creating a standard curve.

**NOTICE**

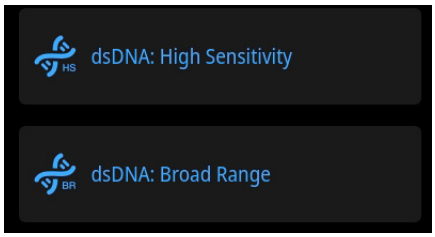
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Sample and standard preparation

For instructions on the preparation of the assay standards and samples, see the instructions that accompany the assay you are using.

## Measure nucleic acid standards and samples using fluorescence

1. From the home screen, select the Fluorescence tab and select **dsDNA Fluorescence** or **RNA Fluorescence**, depending on the samples to be measured.
2. When working with dsDNA, select from one of the assay options. The dsDNA Fluorescence application has both a **High Sensitivity** and a **Broad Range** option which have different dynamic ranges. The RNA Fluorescence application only has a High Sensitivity option, so selection is not needed.




3. Configure any of the setup options if desired and select **Save**.
4. Measure standards (skip this step if using a previous standard curve).

**Note** After the standards are measured the Sample to Reaction Volume option can be changed for each sample by selecting at the top of the screen if desired.

   a. Pipette 2 µL of the prepared assay tube containing Standard #1 onto the lower pedestal and lower the arm.
      - If Auto-Measure is On, the measurement will begin automatically after the arm is lowered.
      - If Auto-Measure is Off, lower arm and select **Measure** to begin the measurement.

**Note** Always use a fresh 2 µL aliquot for each measurement.

b. After the measurement is complete, remove sample from both upper and lower pedestal using a dry laboratory wipe.

c. If Replicates setting is greater than 1, repeat measurement until all replicates are complete.

d. Repeat sub-steps above for Standard #2 including additional replicate measurements.

e. When both standards and all replicates have been measured, a message will appear with options to remeasure standards or begin measuring samples.

f. If finished measuring standards, select **Measure Samples**.

5. Measure samples.

a. Enter or edit the sample ID at the top of the screen by selecting the box and inputting a new sample name.

b. Pipette 2 µL of the assay tube containing the corresponding sample onto the lower pedestal and lower the arm.

- If Auto-Measure is On  , the measurement will begin automatically after the arm is lowered.
- If Auto-Measure is Off  , lower arm and select **Measure** to begin the measurement.

**Note** Always use a fresh 2 µL aliquot for each measurement.

c. When the sample measurement is completed, the reported values are displayed (see the next section).

d. After the measurement is complete, remove sample from both upper and lower pedestal using a dry laboratory wipe.

e. Repeat sub-steps to measure all remaining samples (following the on-screen prompts).

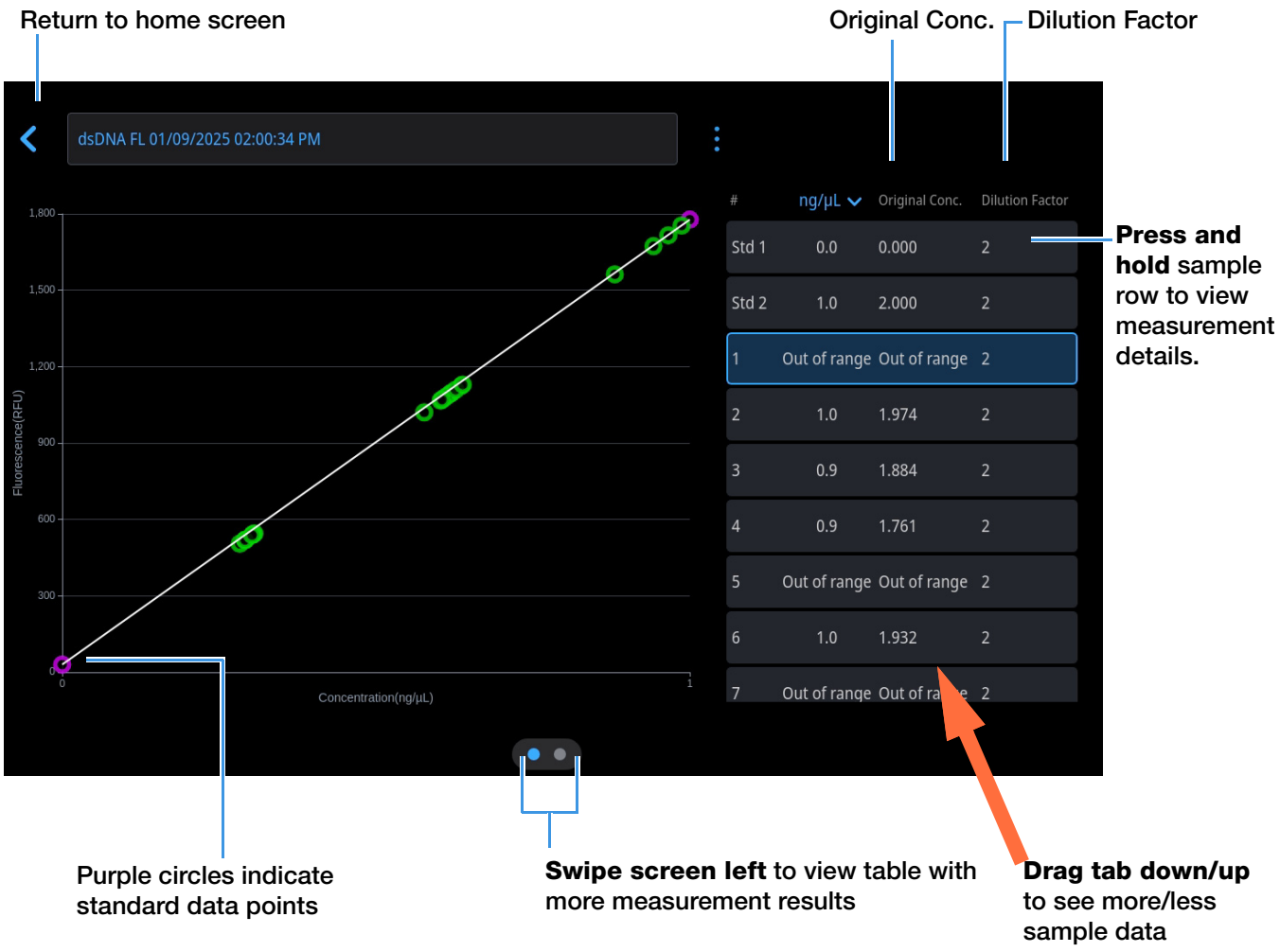
f. When you are finished measuring samples, select **End Experiment**.

6. Lift the arm and clean both pedestals with a new wipe.

# Reported Results

## dsDNA Fluorescence measurement screen (local control)

For each measured sample, the dsDNA Fluorescence and RNA Fluorescence applications show the fluorescence standard curve and a summary of the results. The following is an example of the measurement screen of the NanoDrop™ Ultra local control software screen as seen from History:

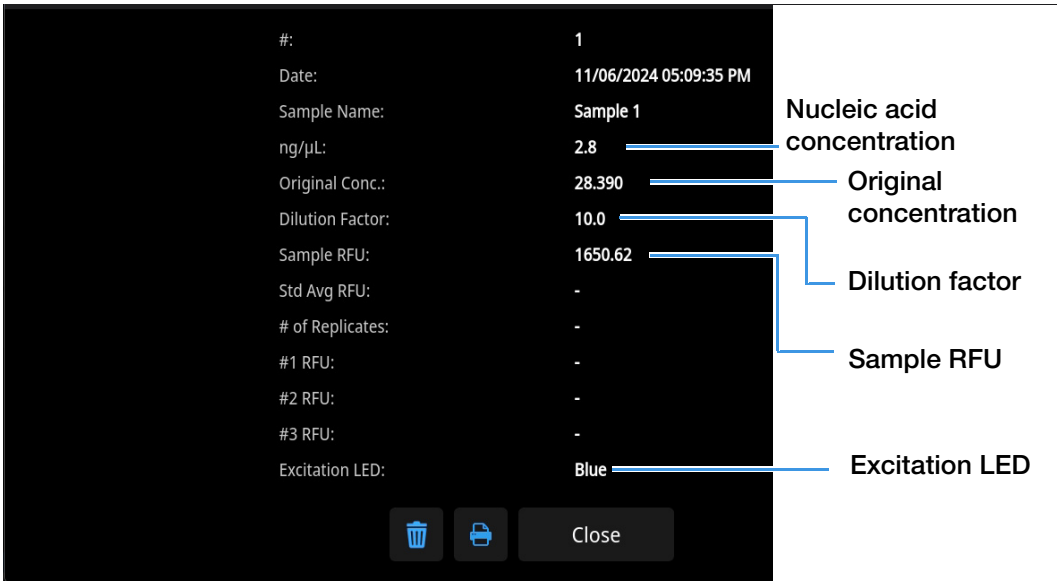


## dsDNA Fluorescence measurement screen (PC control)

For each measured sample, the dsDNA Fluorescence and RNA Fluorescence applications show the fluorescence standard curve and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## dsDNA Fluorescence and RNA Fluorescence reported values

In the local control software, the initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

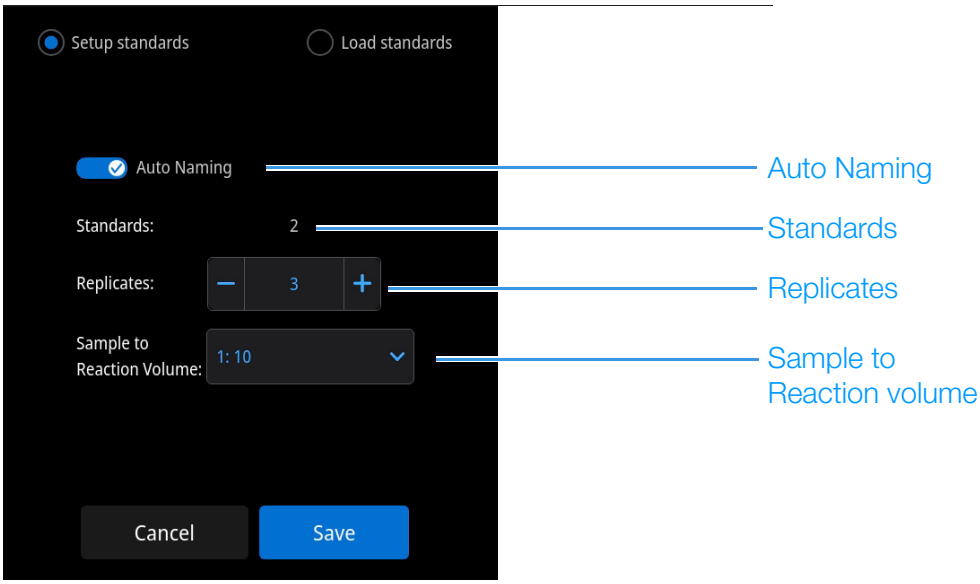



- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- original concentration
- dilution factor
- sample RFU
- excitation LED

# Settings

The dsDNA or RNA Fluorescence Setup screen appears after you select the respective application from the Fluorescence tab on the home screen.

To show the dsDNA Fluorescence or RNA Fluorescence settings from the respective measurement screen, select the Settings icon, .




| Setting | Description |
|---|---|
| Auto Naming | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Standards | This setting cannot be adjusted, all current assays require the use of 2 standards to generate the standard curve. |
| Replicates | Adjust the number of measurements of the same standard that are averaged together to produce its associated RFU value. Up to 5 replicate measurements can be utilized.<br><br>**Note:** Replicates setting cannot be changed after the first standard has been measured. |
| Sample to Reaction Volume | Select from the drop-down menu of available options that reflect the amount of sample in comparison to the total reaction volume. Each assay has 3 available options to select from. See the assay protocol for more details on which option to select. |

# Calculations

A curve-fitting algorithm which requires two standards is used in the calculation of concentration data. The NanoDrop Ultra dsDNA and RNA fluorescence assays use a modified Hill plot to generate concentration data based on the relationship between the two standards used.

Concentration data generated within the fluorescence applications are based on the relationship between the two standards used in creation of the standard curve.

**Measured Values**

**Sample RFU**. Sample RFU refers to the measurement of Relative Fluorescence Units from the given sample, which quantifies the fluorescence intensity emitted by the sample.

**RFU #1**. The Relative Fluorescence Units measured from the first standard replicate.

**RFU #2**. The Relative Fluorescence Units measured from the second standard replicate.

**RFU #3**. The Relative Fluorescence Units measured from the third standard replicate

## Reported Values

**Nucleic acid concentration.**

Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). The sample RFU is used to extrapolate the concentration of the sample from a standard curve (modified hill plot).

**Original nucleic acid concentration.**

The measured concentration and dilution factor are used to calculate the concentration of the original sample before it was added to the assay.

**Dilution factor.**

Ratio of the volume of the original sample to the total volume in the assay tube.

**Standard average RFU.**

Average of the measured RFU for all replicate measurements of a standard.

**# of replicates.**

Number of replicate measurements of each standard.

**Excitation LED.**

Excitation light source used during the measurement.

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# Fluorometer

Choose the excitation light source (blue LED or red LED) while reading fluorescence in both the green and far-red emission channels (for the blue LED) or in the far-red emission channel only (for the red LED). This application is only available for use on the NanoDrop Ultra FL and NanoDrop Ultra$^{C}$ FL and is not compatible with the standard cuvette mode.

Measure Fluorophore-Labeled Samples

Reported Results

Settings

Detection Limits

Calculations

## Measure Fluorophore-Labeled Samples

Use the Fluorometer application to measure the fluorescence emitted by a variety of samples that have been labeled with fluorophore. This application functions similarly to the dsDNA Fluorescence and RNA Fluorescence applications, using a generated standard curve to determine concentration of the measured samples. This application allows for the use of additional assays outside of the NanoDrop Ultra BR and HS Fluorescence Assays.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Sample and standard preparation

For instructions on the preparation of the assay standards and samples, see the instructions that accompany the assay you are using.

See section Working with standard curves for more information on the options available when creating a standard curve.

## Measure fluorophore-labeled standards and samples using fluorescence

1. From the home screen, select the **Fluorescence** tab, then select **Fluorometer**.
2. Select from one of the excitation LED options (Blue or Red).


3. Configure any of the setup options if desired and select **Save**.
4. Measure standards (skip step if using a previous standard curve):
   a. Pipette 2 µL of the prepared assay tube containing the Reference onto the lower pedestal and lower the arm. The reference should contain none of the analyte of interest.
      - If the Auto-Measure feature is OFF  , select **Measure** to begin the measurement.
      - If the Auto-Measure feature is ON  , the measurement will begin automatically after the arm is lowered.

**Note** Always use a fresh 2 µL aliquot for each measurement.

   b. After the measurement is complete, remove sample from both upper and lower pedestal using a dry laboratory wipe.
   c. If Replicates setting is greater than 1, repeat measurement until all replicates are complete.
      – Repeat sub-steps above for Standard #1 and all remaining standards including additional replicate measurements of each.
      – When all standards and all replicates have been measured, a message will appear with options to remeasure standards, load more standards or begin measuring samples.
      – If finished measuring standards, select **Measure Samples**.

5. Measure samples:
   a. Enter or edit the sample ID at the top of the screen by selecting the box and inputting a new sample name.
   b. Pipette 2 µL of the assay tube containing the corresponding sample onto the lower pedestal and lower the arm.
      - If the Auto-Measure feature is OFF  , select **Measure** to begin the measurement.
      - If the Auto-Measure feature is ON  , the measurement will begin automatically after the arm is lowered.

**Note** Always use a fresh 2 µL aliquot for each measurement.

   c. When the sample measurement is completed, the standard curve and reported values are displayed (see the next section).
   d. After the measurement is complete, remove sample from both upper and lower pedestal using a dry laboratory wipe.
   e. Repeat sub-steps to measure all remaining samples (following the on-screen prompts).
   f. When you are finished measuring samples, select **End Experiment**.
6. Lift the arm and clean both pedestals with a new wipe.

## Related Topics

- Best Practices for Nucleic Acid Measurements
- Measure a Micro-Volume Sample
- Best Practices for Micro-Volume Measurements
- Basic Instrument Operations

# Reported Results

## Fluorometer measurement screen (local control)

For each measured sample, the Fluorometer application shows the fluorescence standard curve and a summary of the results. Here is an example of the NanoDrop Ultra local control software screen:


**Tap** to end experiment

Sample name; **tap** to edit

Settings

Nucleic acid concentration

**Tap** to select unit

Original concentration

Dilution factor

**Press and hold** sample row to view measurement details.

**Drag tab down/up** to see more/less sample data

Auto-Measure

Green circles indicate sample data points

**Swipe screen left** to view table with more measurement results

Purple circles indicate standard data points

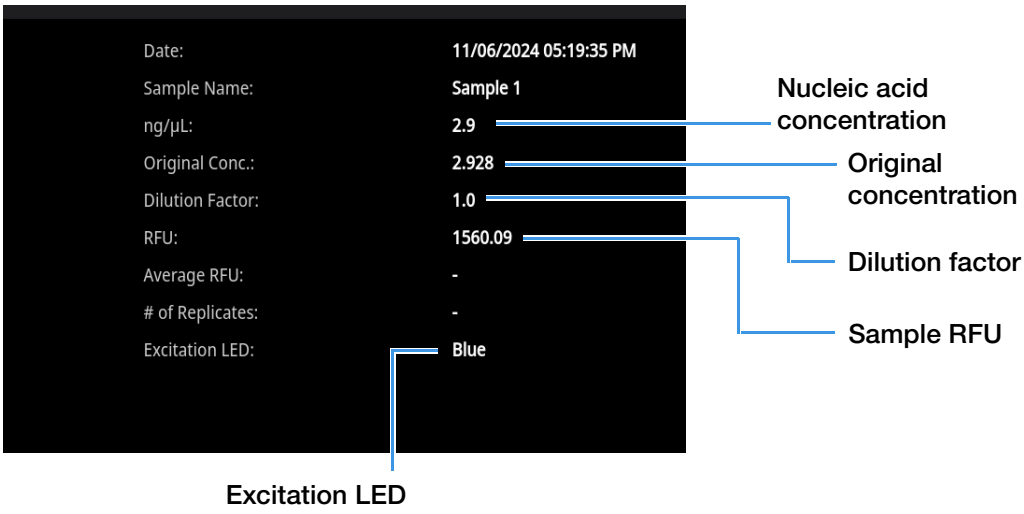
**Tap** to end experiment and export data

## Fluorometer measurement screen (PC control)

For each measured sample, this application shows the fluorescence standard curve and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Reported values

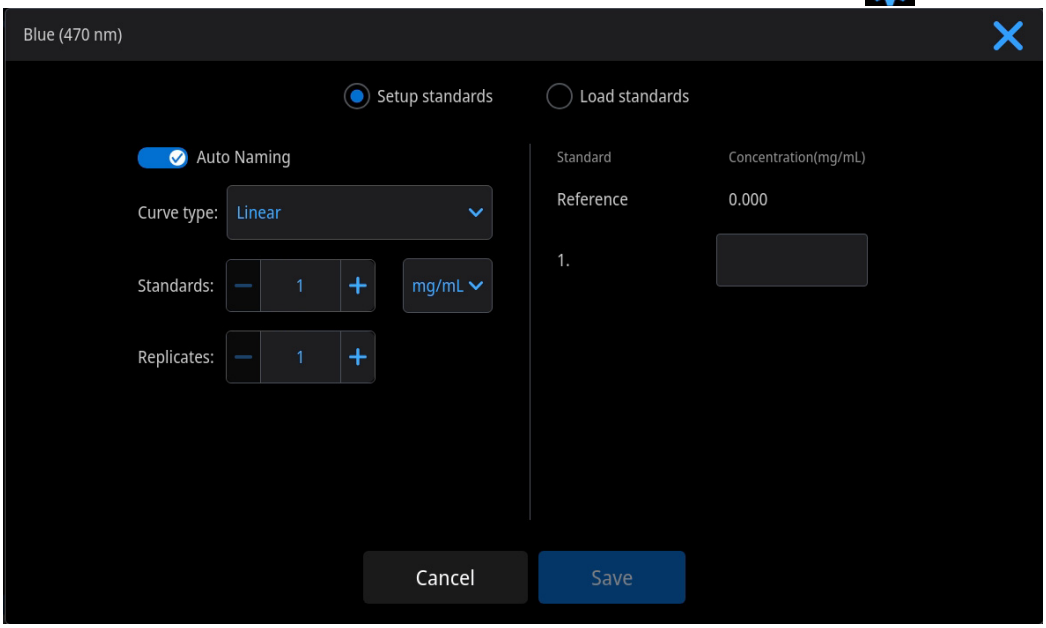
In the local control software, the initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




- date (date and time sample measurement was taken)
- sample name
- analyte concentration
- RFU
- Extraction LED

## Settings

The Fluorometer Setup screen appears after you select the Fluorometer application from the Fluorescence tab on the home screen. To show the Fluorometer settings from the Fluorometer measurement screen, select the Settings icon, .



| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Curve Type | | Specify type of equation used to create standard curve from standard concentration values.<br><br>Available options:<br>– **Linear**: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard)<br>– **2nd order polynomial**: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards)<br>– **3rd order polynomial**: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards) |
| Replicates | | Enter number of duplicate measurements of the reference or the same standards that are then averaged together to produce its associated concentration value.<br><br>**Note**: Replicates setting cannot be changed after the first standard has been measured. |
| Standards | | Enter actual concentration value of each standard and select the concentration units.<br><br>**Note**: Concentration values can be entered in any order but the standards must be measured in the order they were entered.<br><br>If you also want to use previously measured standards, select **Load Standards**. |

# Reagent Calculator

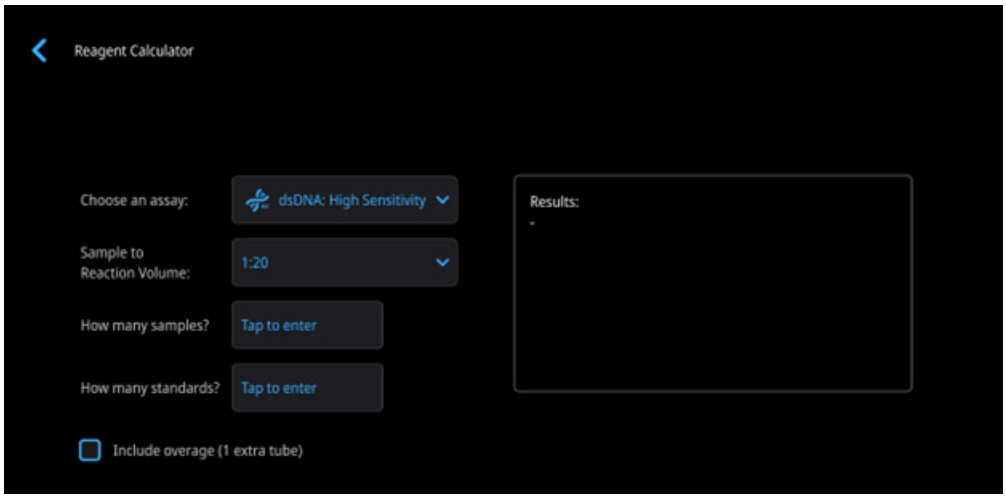
Calculates the necessary volume of each NanoDrop Ultra Fluorescence Assay component (dye and buffer) required to make the appropriate amount of working solution needed to prepare your samples and standards.

Use Reagent Calculator

Results

## Use the Reagent Calculator

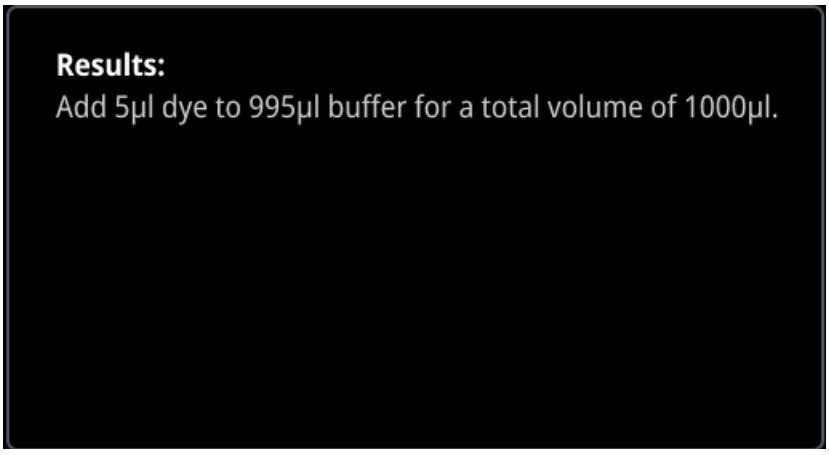
1. From the home screen, select the **Fluorescence** tab, then select **Reagent Calculator**.
2. Select one of the available assays from the drop-down menu next to **Choose an assay**.
3. Select one of the available **Sample to Reaction Volume** options for the assay chosen above.
4. See the protocol provided with your assay for more information on which Sample to Reaction Volume is best for your samples.
5. Select the total number of samples and standards that will be measured by selecting each respective box and entering the desired amount.
6. Optional: Enable **Include overage** (1 extra tube) by selecting the empty check box to include an additional tube into the calculation to ensure enough working solution is created for all samples and standards.



# Reagent Calculator Results

When all fields are populated the Results: window will display the necessary volume of each NanoDrop™ Ultra Fluorescence Assay component (dye and buffer) to create the necessary amount of working solution for the number of samples and standards selected. The total volume of the working solution created will also be displayed.

The calculator uses a 1:200 ratio of dye/reagent to buffer required by the NanoDrop™ Ultra Fluorescence Assays.



# Acclaro Pro Applications

New versions of the most popular measurement applications can be unlocked providing as little as ± 5% error throughout the absorbance dynamic range. These applications are not compatible with the standard cuvette mode. Users may notice an increased measurement time required to achieve the higher level of accuracy compared to the standard applications.

## Activating Acclaro Pro

Thermo Scientific™ Acclaro™ Pro is an optional software add-on. If you have not purchased this add-on and are interested in using these applications, please purchase it at thermofisher.com/nanodrop or reach out to your local sales representative. The NanoDrop Ultra Spectrophotometers and Fluorometers will need to be serviced to unlock the Acclaro Pro applications.

## dsDNA, ssDNA or RNA Pro

More accurately measures the concentration of highly concentrated (> 62.5 A) purified dsDNA, ssDNA or RNA samples that absorb at 260 nm using the Acclaro Pro enhanced automated pathlength selection algorithm.


Measure dsDNA Pro, ssDNA Pro, or RNA Pro

Reported Results

Settings

Detection Limits

Calculations

# Best practices for nucleic acid measurements

- Isolate and purify nucleic acid samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA, free nucleotides, proteins, some buffer components and dyes. See Preparing Samples for more information.

  **Note** Extraction reagents such as guanidine, phenol, and EDTA contribute absorbance between 230 nm and 280 nm and will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 260 nm), you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - If possible, heat highly concentrated or large molecule samples, such as genomic or lambda DNA, to 63 °C (145 °F) and gently (but thoroughly) vortex before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.

## Related Topics

- Measure a Micro-Volume Sample
- Best Practices for Micro-Volume Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Measure dsDNA Pro, ssDNA Pro or RNA Pro

Use the dsDNA Pro, ssDNA Pro and RNA Pro applications to quantify purified double-stranded (ds) or single-stranded (ss) DNA or RNA samples with increased accuracy at higher concentrations (> 62.5 A). These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

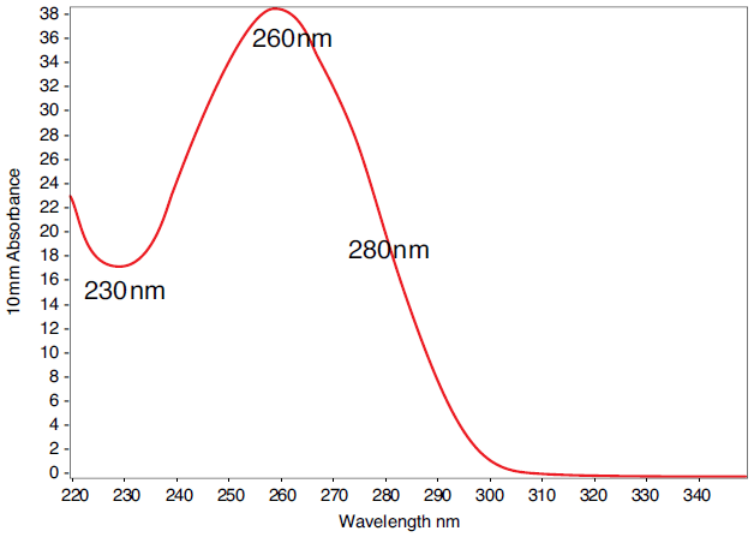
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

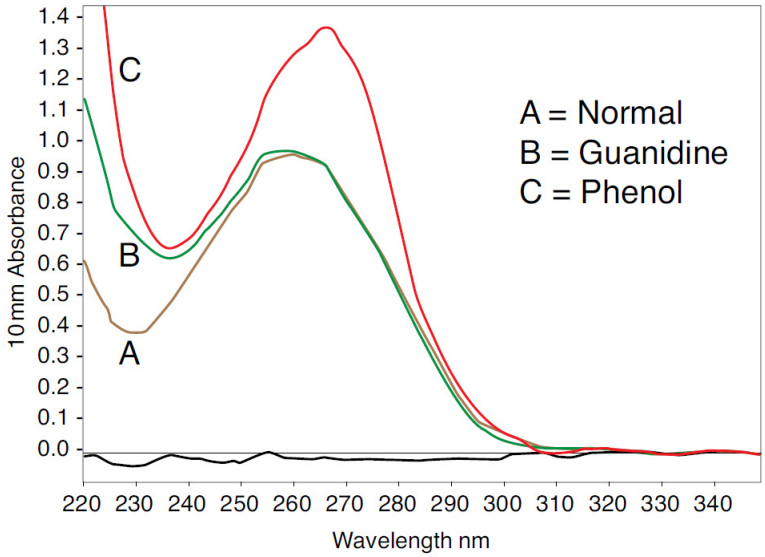
1. From the home screen, select the **Acclaro Pro** tab, then select **dsDNA Pro**, **ssDNA Pro** or **RNA Pro**, depending on the samples to be measured.
2. Configure any of the setup options if desired and select **Save**.
3. Pipette 1-2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.
5. Lift the arm and clean both pedestals with a new laboratory wipe.
6. Pipette 1-2 µL sample solution onto the pedestal.
7. Start the sample measurement:
   - If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe.




**Typical nucleic acid spectrum**

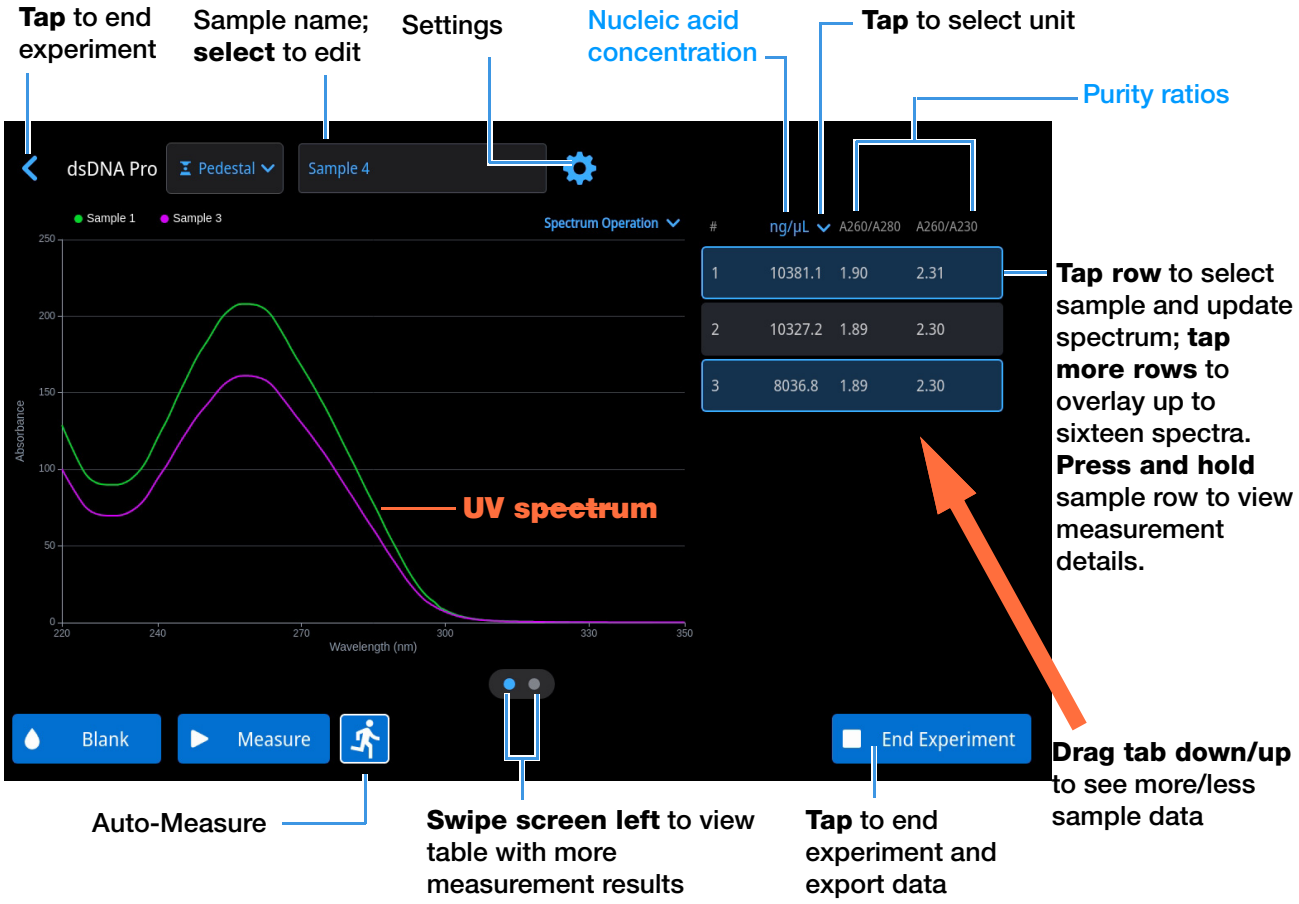



**Comparison of nucleic acid spectra with and without two common contaminants**

# Reported Results

## dsDNA Pro measurement screen (local control)

For each measured sample, the dsDNA Pro, ssDNA Pro and RNA Pro applications show the UV absorbance spectrum and a summary of the results. Below is an example of the measurement screen of the NanoDrop Ultra local control software:



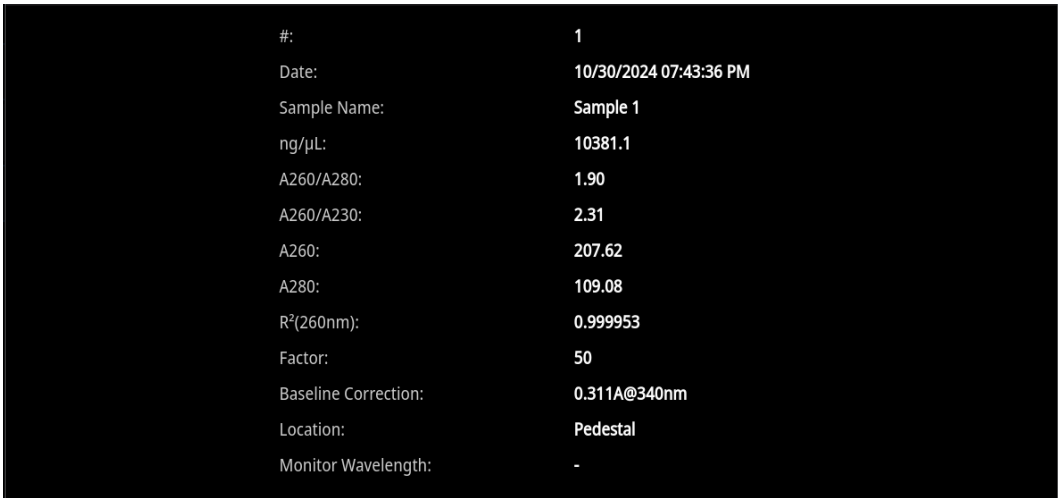

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## dsDNA Pro measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- R2 (260nm)
- A280
- factor
- baseline correction
- location
- monitor wavelength

## Settings

The dsDNA Pro, ssDNA Pro, or RNA Pro Setup screen appears after you select the respective application from the Acclaro Pro tab on the home screen. To show the dsDNA Pro, ssDNA Pro or RNA Pro settings, from the dsDNA Pro, ssDNA Pro or RNA Pro measurement screen, select  .

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. When enabled, the software corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

# Calculations

The Acclaro Pro Nucleic Acid applications use a modification of the Beer-Lambert equation (shown at right) to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

### Extinction Coefficients vs Factors

Using the terms in the Beer-Lambert equation, factor (f) is defined as:

$$\text{factor (f)} = 1/(\varepsilon * b)$$

where:
$\varepsilon$ = wavelength-dependent molar extinction coefficient in ng-cm/µL
b = **sample pathlength** in cm

As a result, analyte concentration (c) is calculated as:

$$c = A * [1/(\varepsilon * b)]$$

or

$$c = A * f$$

where:
c = analyte concentration in ng/µL
A = absorbance in absorbance units (A)
**f** = factor in ng-cm/µL (see below)

For the dsDNA Pro, ssDNA Pro and RNA Pro applications, the generally accepted factors for nucleic acids are used in conjunction with Beer's Law to calculate sample concentration. For the Custom Factor Pro application, the user-specified factor is used.

### Factors Used

- **dsDNA** (factor = 50 ng-cm/µL)
- **ssDNA** (factor = 33 ng-cm/µL)
- **RNA** (factor = 40 ng-cm/µL)
- **Custom Factor** (user entered factor between 15 ng-cm/µL and 150 ng-cm/µL

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

**Note**: For micro-volume absorbance measurements, the spectra are normalized to a 10 mm pathlength equivalent.

### A260 absorbance

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum with an improved algorithm. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

### A230 and A280 absorbance

- Normalized and baseline-corrected (if selected) absorbance values at 230 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

### Reported Values

- **Nucleic acid concentration**. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~1.8 is generally accepted as "pure" for DNA (~2.0 for RNA). Acidic solutions may under represent the reported value by 0.2-0.3; the opposite is true for basic solutions.
- **A260/A230 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm. An A260/A230 purity ratio between 1.8 and 2.2 is generally accepted as "pure" for DNA and RNA.

**Note**: Although purity ratios are important indicators of sample quality, the best quality indicator quality is functionality in the downstream application of interest (e.g., real-time PCR).

- **A260 absorbance**.
- **A280 absorbance**.
- **$R^2$ (260nm)**. Coefficient of determination for the line of best fit for 260 nm measurements.
- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays that the measurement was taken from the pedestal mode.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.

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# Custom Factor Pro

More accurately measures the concentration of highly concentrated (> 62.5 A) purified nucleic acids using a custom factor and the Acclaro Pro enhanced automated pathlength selection algorithm.




## Measure Custom Factor Pro

Use the Custom Factor application to quantify purified DNA or RNA samples with increased accuracy at higher concentrations (> 62.5 A) that absorb at 260 nm with a user-defined extinction coefficient or factor. The application reports nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

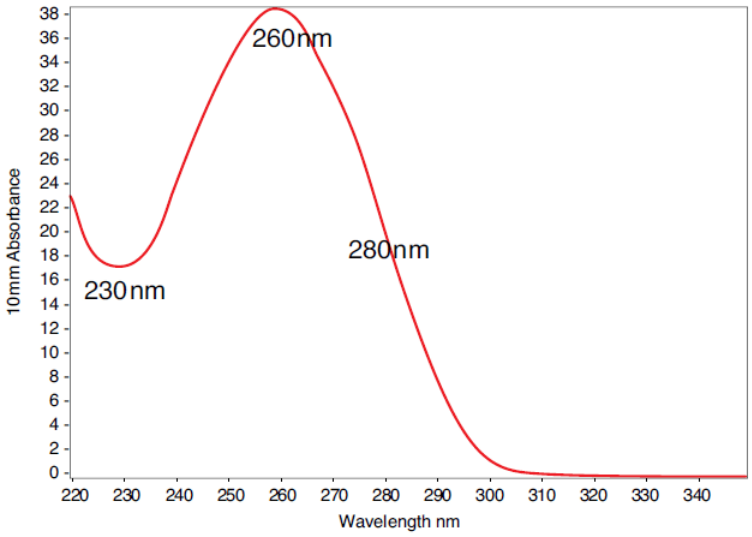
### Procedure

1. From the home screen, select the **Acclaro Pro** tab, then select **Custom Factor Pro**.

2. Configure any of the setup options if desired and select **Save**.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.
5. Lift the arm and clean both pedestals with a new laboratory wipe.
6. Pipette 1-2 µL sample solution onto the pedestal.
7. Start the sample measurement:
   - If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe.




**Typical nucleic acid spectrum**

## Related Topics

- Measure a Micro-Volume Sample
- Best Practices for Micro-Volume Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Custom Factor Pro measurement screen (local control)

For each measured sample, the Custom Factor Pro application shows the absorbance spectrum and a summary of the results. Here is an example of the measurement screen of the NanoDrop Ultra local control software:

**Note** The Custom Factor Pro measurement screen is identical to the measurement screen for the other standard nucleic acid Pro applications.


## Custom Factor Pro measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

# Settings

The Custom Factor Pro Setup screen appears after you select the application from the Acclaro Pro tab on the home screen. To show the Custom Factor Pro settings from the Custom Factor Pro measurement screen, select  .

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name "sample" followed by the number sample in the sequence. For example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name. |
| Custom Factor | Enter an integer value between 15 ng-cm/µL and 150 ng-cm/µL | Constant used to calculate nucleic acid concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength:<br><br>$f = 1/(\varepsilon_{260} * b))$<br><br>where:<br>**f**= factor<br>$\varepsilon$ = molar extinction coefficient at 260 nm in ng-cm/µL<br>**b** = sample pathlength in cm (1 cm for nucleic acids measured with the NanoDrop Ultra instruments) |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | **Optional user-defined baseline correction**. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

# Detection Limits

The lower detection limits and reproducibility specifications for nucleic acids are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the user-defined extinction coefficients.

## To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

### Related Topics

- Detection Limits for All Applications

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# Oligo DNA Pro or Oligo RNA Pro

More accurately measures the concentration of highly concentrated (> 62.5 A) purified ssDNA or RNA oligonucleotides that absorb at 260 nm using the Acclaro Pro enhanced automated pathlength selection algorithm.




## Measure Oligo DNA Pro or Oligo RNA Pro

Use the Oligo DNA Pro and Oligo RNA Pro applications to quantify oligonucleotides that absorb at 260 nm with increased accuracy at higher concentrations (> 62.5 A). Molar extinction coefficients are calculated automatically based on the user-defined base sequence of the sample. These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** If the oligonucleotide has been modified, for example with a fluorophore dye, check with the oligo manufacturer to determine if the modification contributes absorbance at 260 nm. If it does, we recommend using the Microarray application to quantify nucleic acid concentration. The Microarray application includes a correction to remove any absorbance contribution due to the dye from the oligo quantification result.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

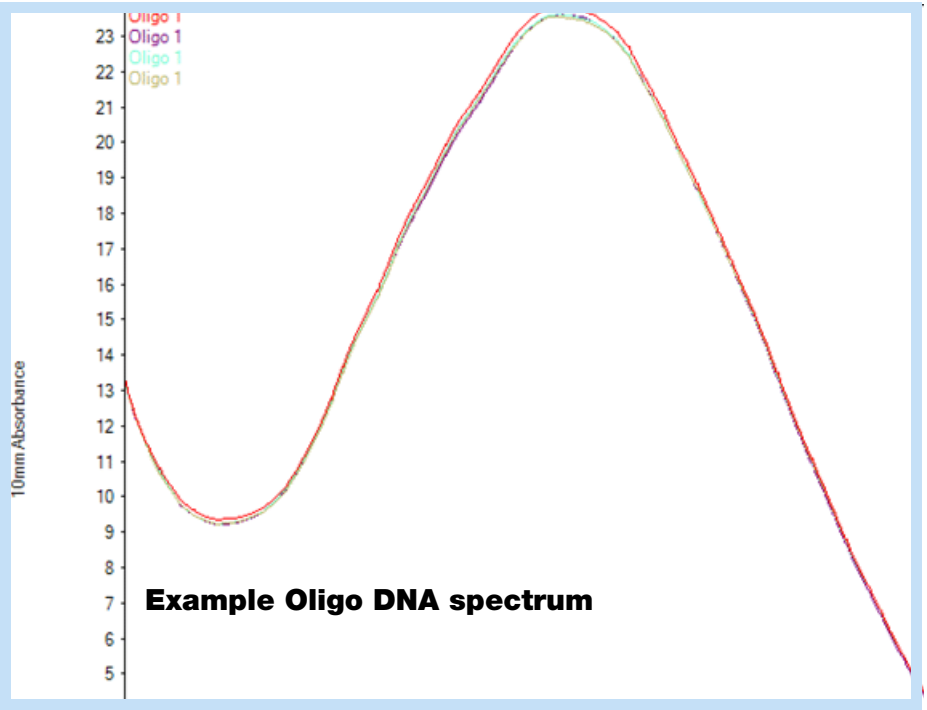
## Procedure

1. From the home screen, select the **Acclaro Pro** tab, then select either **Oligo DNA Pro** or **Oligo RNA Pro**, as needed.
2. Configure any of the setup options if desired and select **Save**.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.
5. Lift the arm and clean both pedestals with a new laboratory wipe.
6. Pipette 1-2 µL sample solution onto the pedestal.
7. Start the sample measurement:
   – If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe.



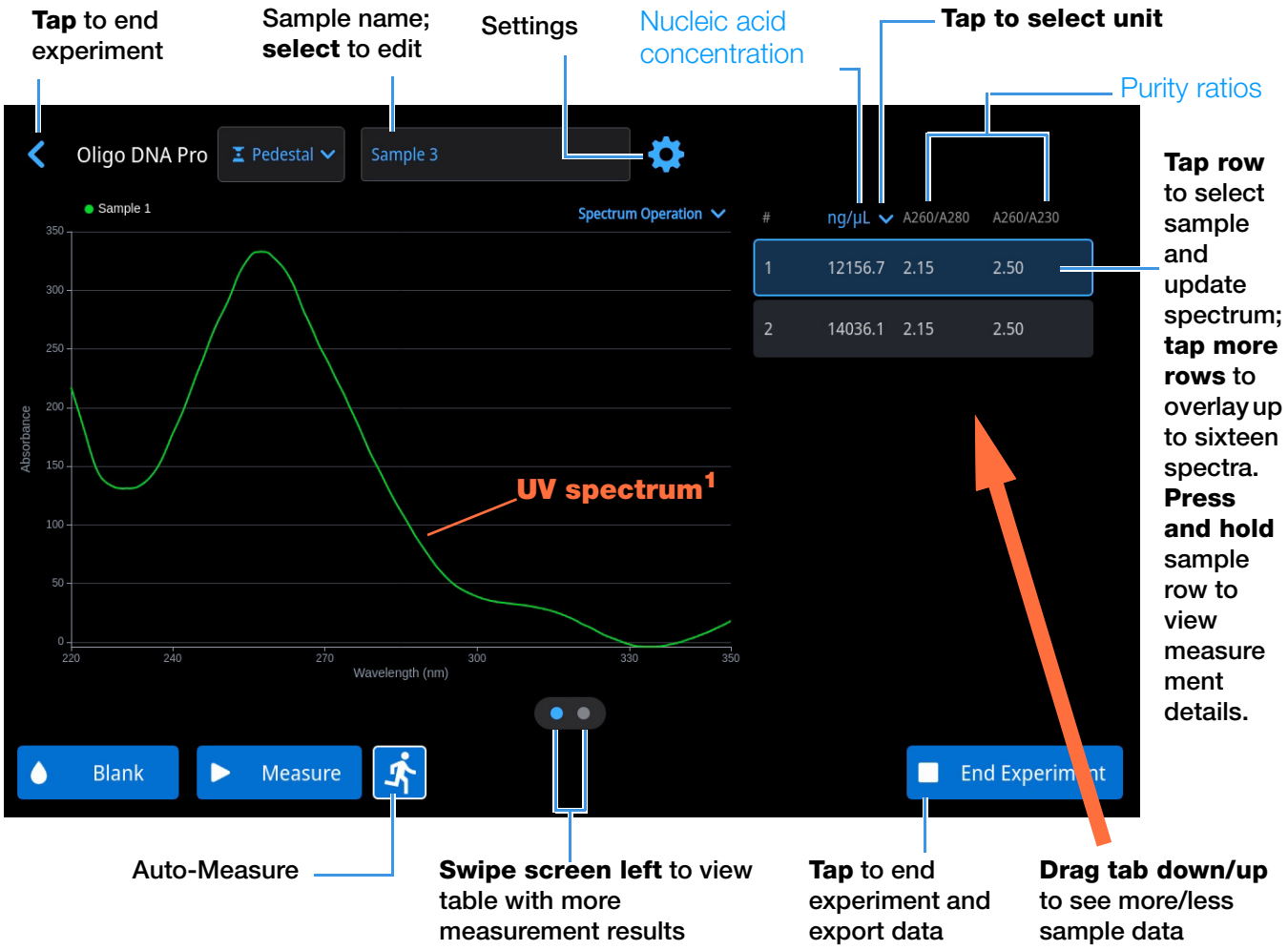

## Related Topics

- Best Practices for Nucleic Acid Measurements
- Measure a Micro-Volume Sample
- Best Practices for Micro-Volume Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Oligo DNA Pro and Oligo RNA Pro measurement screen (local control)

For each measured sample, the Oligo DNA Pro and Oligo RNA Pro applications show the UV absorbance spectrum and a summary of the results. Here is an example of the measurement screen of the NanoDrop Ultra local control software:




[1]Measured oligo: TTT TTT TTT TTT TTT TTT TTT TTT

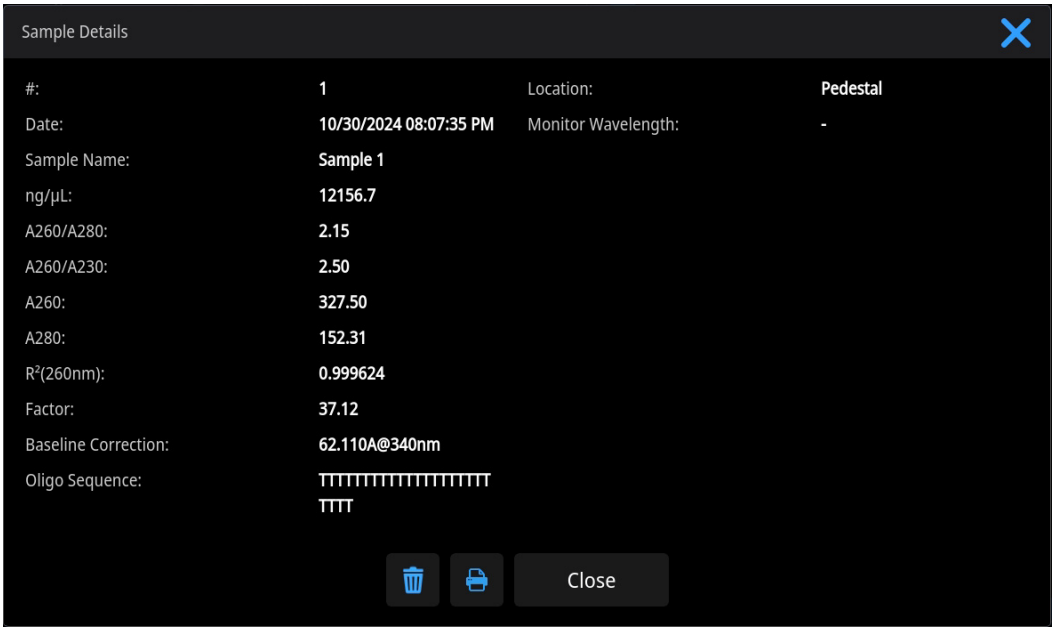
**Note** Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

## Oligo DNA Pro and Oligo RNA Pro measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- R2 (260nm)
- factor

- baseline correction
- oligo sequence
- location
- pathlength used
- monitored wavelength

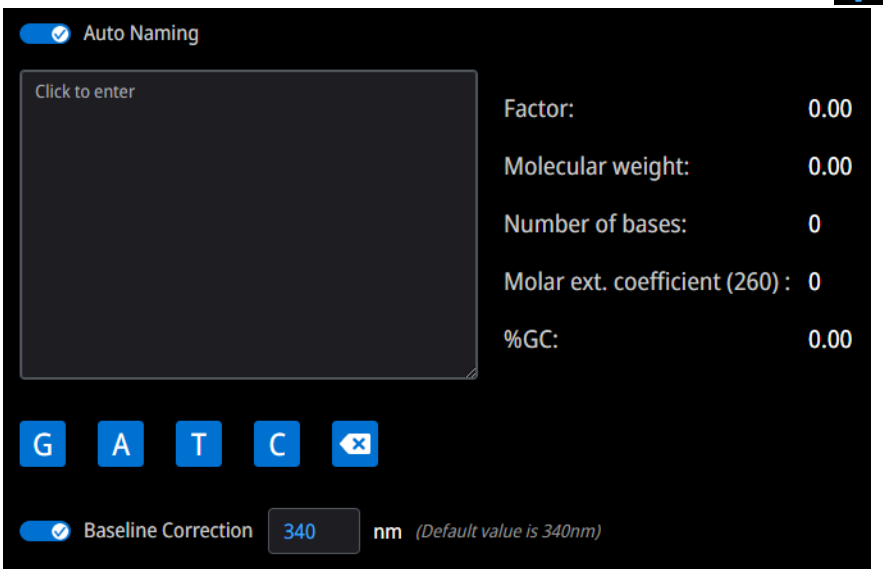
**Note** The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information.

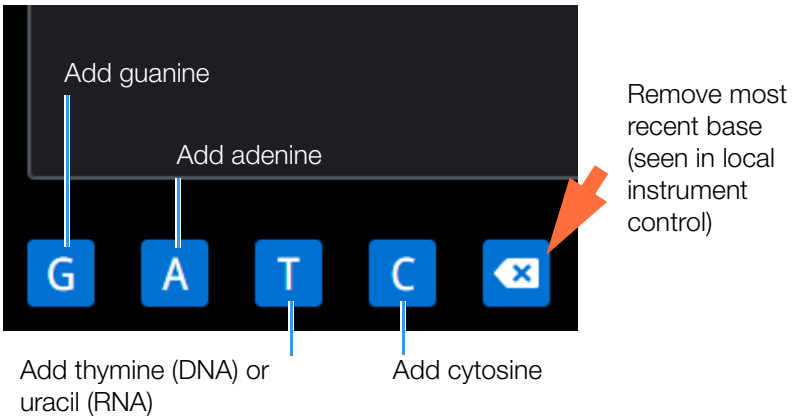
### Related Topics

- Basic Instrument Operations
- Oligo Calculations

## Settings

The Oligo DNA Pro or Oligo RNA Pro Setup screen appears after you select the respective application from the Acclaro Pro tab on the home screen. To show the Oligo Pro settings from the Oligo Pro measurement screen, select .



| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Oligo Base Sequence | for DNA: Use the G, A, T and C keys to specify the DNA base sequence<br><br>for RNA: Use the G, A, U and C keys to specify the RNA base sequence | Specify your DNA or RNA base sequence.<br>Tap or click the corresponding keys:<br>  <br><br>From the PC control software, you can also enter base sequence using the keyboard, or by copy and pasting a sequence from another application. |
| | | Each time a base is added to the sequence, the software calculates the following:<br><br>• **Factor**. Constant used to calculate oligonucleotide concentration in modified Beer’s Law equation. Based on extinction coefficient and pathlength:<br><br>$f = 1/(\varepsilon_{260} * b)$<br><br>where:<br>**f**= factor<br>$\varepsilon$ = molar extinction coefficient at 260 nm in ng-cm/µL<br>**b** = sample pathlength in cm (0.1 cm for nucleic acids measured with the NanoDrop Ultra instrument) |

| Setting | Available Options | Description |
|---|---|---|
| | | • **Molecular Weight** of oligo calculated from user-defined base sequence.<br>• **Number of Bases** entered.<br>• **Molar Ext. Coefficient (260 nm)**. Molar extinction coefficient of oligo (in ng-cm/µL) at 260 nm calculated from entered base sequence.<br>• **%GC**. Percentage of guanine and cytosine residues in total number of bases entered. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction. |

### Related Topics

- Instrument Settings

## Detection Limits

The lower detection limits and reproducibility specifications for the oligonucleotide sample types (ssDNA and RNA) are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the extinction coefficients for the user-defined base sequences.

### To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

# Calculations

As with the other nucleic acid applications, the Oligo Pro applications use the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength. Because oligonucleotides are short, single-stranded molecules (or longer molecules of repeating sequences), their spectrum and extinction coefficient ($\varepsilon$) are closely dependent on base composition and sequence.

(The generally accepted extinction coefficients and factors for single-stranded DNA and RNA provide a reasonable estimate for natural, essentially randomized, sequences but not for short, synthetic oligo sequences.) To ensure the most accurate results, we use the exact value of $\varepsilon_{260}$ to calculate oligonucleotide concentration.

The NanoDrop software allows you to specify the base sequence of an oligonucleotide before it is measured. For any entered base sequence, the software uses the equation at the right to calculate the extinction coefficient.

**Tip**: The extinction coefficient is wavelength specific for each oligonucleotide and can be affected by buffer type, ionic strength and pH.

## Extinction Coefficients for Oligonucleotides

The software uses the nearest neighbor method and the following formula to calculate molar extinction coefficients for specific oligonucleotide base sequences:

$$\varepsilon_{260} = \sum_{1}^{N-1} \varepsilon_1 - \sum_{2}^{N-1} \varepsilon_2 + \sum_{1}^{N} \varepsilon_3$$

where:
$\varepsilon$ = molar extinction coefficient in L/mole-cm
$\varepsilon_1 = \varepsilon_{\text{nearest neighbor}}$
$\varepsilon_2 = \varepsilon_{\text{individual bases}}$
$\varepsilon_3 = \varepsilon_{\text{modifications, such as fluorescent dyes}}$

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. Estimated A260/A280 ratios for each independently measured nucleotide are provided below:

Guanine: 1.15
Adenine: 4.50
Cytosine: 1.51
Uracil: 4.00
Thymine: 1.47

The A260/A280 ratio for a specific nucleic acid sequence is approximately equal to the weighted average of the A260/A280 ratios for the four nucleotides present.

**Note**: RNA will typically have a higher 260/280 ratio due to the higher ratio of Uracil compared to that of Thymine.

## Measured Values

### A260 absorbance

**Note**: For micro-volume absorbance measurements, the spectra are normalized to a 10 mm pathlength equivalent.

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the sample absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

### A230, A280 absorbance

- Normalized absorbance values at 230 nm, 260 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

## Reported Values

- **Nucleic acid concentration**. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm.
- **A260/A230 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm.

**Note**: The traditional purity ratios (A260/A280 and A260/A230), which are used as indicators of the presence of various contaminants in nucleic acid samples, do not apply for oligonucleotides because the shapes of their spectra are highly dependent on their base compositions. See side bar for more information.

- **A260 absorbance**.
- **A280 absorbance**.
- **$R^2$ (260nm)**. Coefficient of determination for the line of best fit for 260 nm measurements.
- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Oligo Sequence**.
- **Location**. Displays that the measurement was taken from the pedestal.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.

## Related Topics

- Calculations for Nucleic Acid Measurements

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# Protein A280 Pro

More accurately measures the concentration of highly concentrated (> 62.5 A) purified protein samples that absorb at 280 nm using the Acclaro Pro enhanced automated pathlength selection algorithm.


Measure Protein A280 Pro

Reported Results

Settings

Detection Limits

Calculations

## Best practices for protein measurements

- Isolate and purify protein samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA and some buffer components. See Preparing Samples for more information.

  **Note** Extraction reagents that contribute absorbance between 200 nm and 280 nm will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.

- Choosing a blank:

  – For the Protein A280 Pro application, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.

- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 280 nm), you may need to choose a different buffer or application, such as a colorimetric assay (for example, BCA or Pierce 660). See Choosing and Measuring a Blank for more information.

  **Note** Buffers such as Triton X, RIPA, and NDSB contribute significant absorbance and are not compatible with direct A280 measurements.

- For micro-volume measurements:

  – Ensure pedestal surfaces are properly cleaned and conditioned. (Proteins tend to stick to pedestal surfaces.)

- Gently (but thoroughly) vortex samples before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
- Follow best practices for micro-volume measurements.
- Use a 2 µL sample volume. See Recommended Sample Volumes for more information.

### Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Prepare Samples and Blanks
- Basic Instrument Operations

## Measure Protein A280 Pro

Use the Protein A280 application to quantify purified protein samples that contain amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, which exhibit absorbance at 280 nm with increased accuracy at higher concentrations (> 62.5 A). This application reports protein concentration measured at 280 nm and one absorbance ratio (A260/A280). A single-point baseline correction can also be used. This application does not require a standard curve.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** If your samples contain mainly peptide bonds and little or no amino acids, use the Protein A205 application instead of Protein A280 Pro.

**NOTICE**

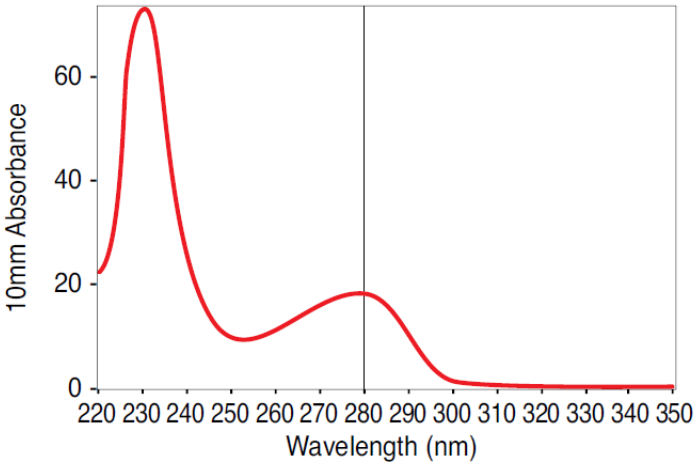
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

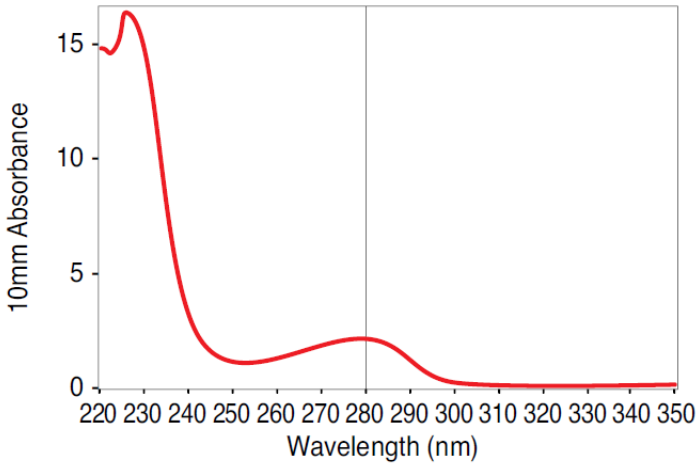
1. From the home screen, select the **Acclaro Pro** tab, then select **Protein A280 Pro**.
2. Configure any of the setup options if desired and select **Save**.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.
5. Lift the arm and clean both pedestals with a new laboratory wipe.
6. Pipette 2 µL sample solution onto the pedestal.
7. Start the sample measurement:
   - If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe.




**High concentration BSA sample**

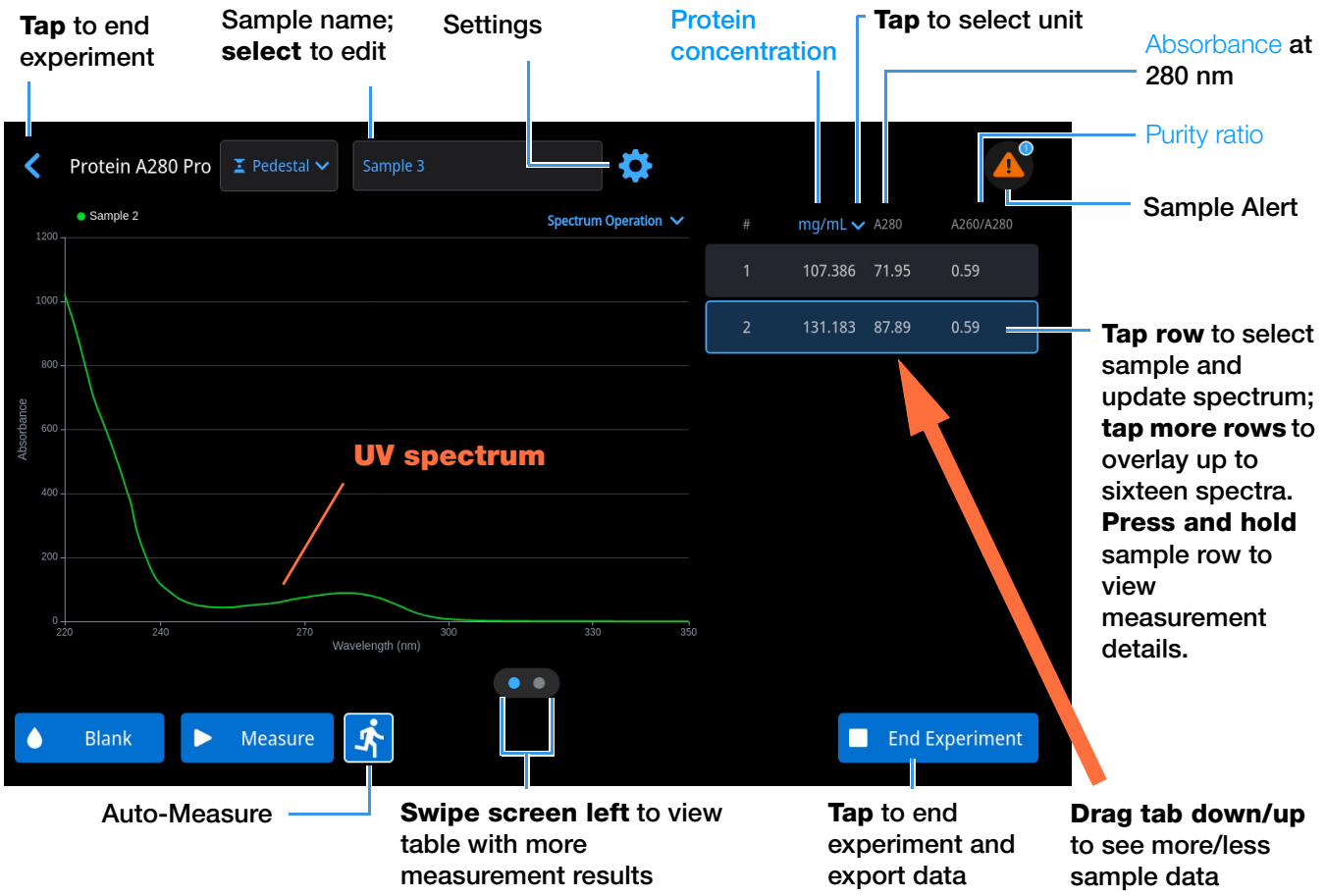



**Low concentration BSA sample**

# Reported Results

## Protein A280 Pro measurement screen (local control)

For each measured sample, the Protein A280 Pro application shows the absorbance spectrum and a summary of the results. Here is an example of the measurement screen within the NanoDrop Ultra local control software:



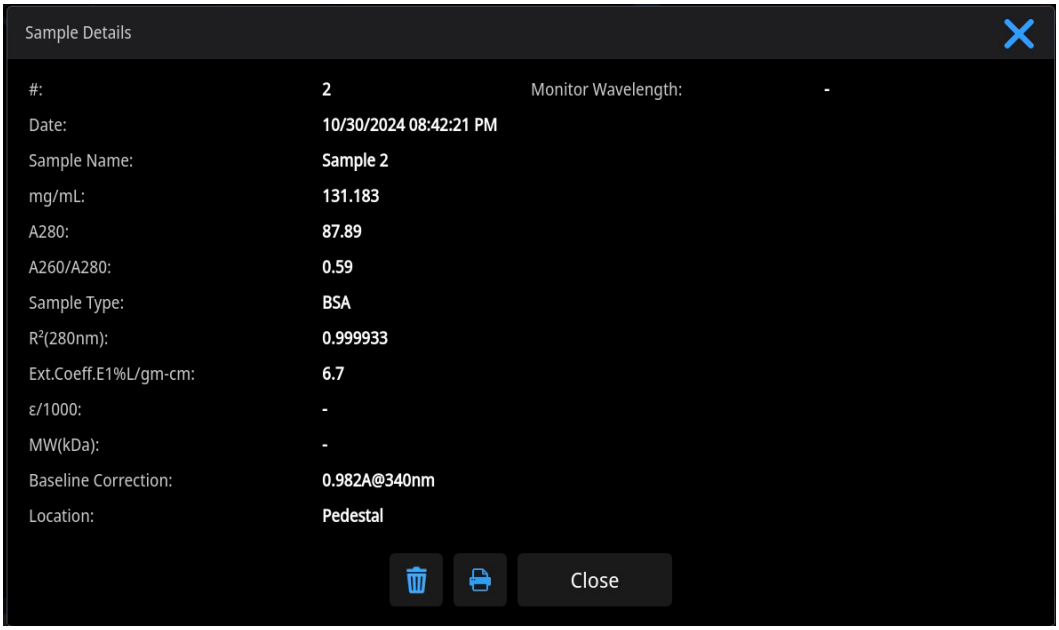

**Note** Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

## Protein A280 Pro measurement screen (PC control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Reported values

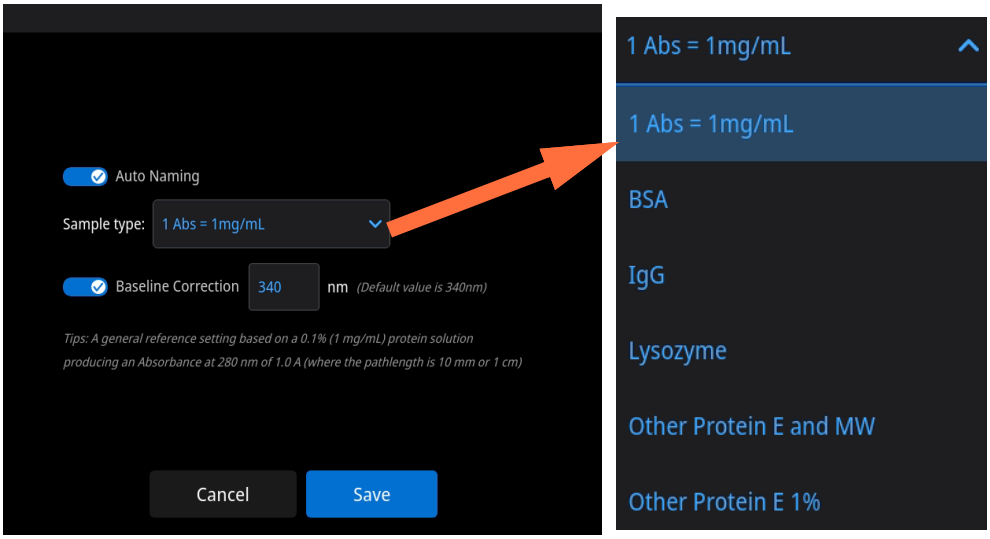
The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- protein concentration
- A280
- A260/A280
- sample type
- R2 (280nm)
- baseline correction
- location
- monitored wavelength
- mass extinction coefficient (1% solution)
- molar extinction coefficient
- molecular weight (kDA)

# Settings

The Protein A280 Pro application provides a variety of sample type options for purified protein analysis.




Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the ε + MW (molar) or ε1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option.

**Tip** Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

The Protein A280 Pro Setup screen appears after you select the application from the Acclaro Pro tab on the home screen. To show the Protein A280 Pro settings from the Protein A280 Pro measurement screen, select .

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| Auto Naming | On or off | N/A | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (340 nm) | N/A | Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.<br><br>**Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction. |
| Sample type[a] | 1 Abs = 1 mg/mL | General reference | Recommended when extinction coefficient is unknown and rough estimate of protein concentration is acceptable for a solution with no other interfering substances. Assumes 0.1% (1 mg/mL) protein solution produces 1.0A at 280 nm (where pathlength is 10 mm), i.e., $\varepsilon 1\% = 10$. |
| | BSA | 6.7 | Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient ($\varepsilon$) of 6.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) BSA solution. Assuming MW is 66,400 daltons (Da), molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$. |

| Setting | Available Options | Mass Ext. Coefficient (L/gm-cm) | Description |
|---|---|---|---|
| | IgG | 13.7 | Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (ε) of 13.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) IgG solution. Assuming MW is 150,000 Da, molar extinction coefficient at 280 nm for IgG is approximately 210,000 $M^{-1}cm^{-1}$. |
| | Lysozyme | 26.4 | Calculates lysozyme protein concentration using mass extinction coefficient (ε) of 26.4 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) lysozyme solution. Assumes molar extinction coefficient for egg white lysozyme ranges between 36,000 $M^{-1}cm^{-1}$ and 39,000 $M^{-1}cm^{-1}$. |
| | Other protein (ε + MW) | User entered molar extinction coefficient and molecular weight | Assumes protein has known molar extinction coefficient (ε) and molecular weight (MW), where:<br>$(\varepsilon_{molar})*10=(\varepsilon_{percent})*(MW_{protein})$<br>Enter MW in kiloDaltons (kDa) and molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (i.e., ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210. |
| | Other protein (ε1%) | User entered mass extinction coefficient | Assumes protein has known mass extinction coefficient (ε). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (ε1%) protein solution. |

[a] To add or edit a custom protein, use Protein Editor.

# Detection Limits

Detection limits and reproducibility specifications for purified BSA proteins are provided here. The BSA lower detection limit and reproducibility values apply to any protein sample type. The upper detection limits are dependent on the upper absorbance limit of the instrument and the sample's extinction coefficient.

### To calculate upper detection limits for other (non-BSA) protein sample types

To calculate upper detection limits in ng/µL for proteins, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} / \text{mass extinction coefficient}_{\text{sample}}) * 10$$

For example, if the sample's mass extinction coefficient at 280 nm is 6.7 for a 1% (10 mg/mL) solution, the equation looks like this:

(550 / 6.7) * 10 = 824.6 (or ~825)

# Calculations

The Protein A280 Pro application uses the Beer-Lambert equation to correlate absorbance with concentration. Solving Beer's law for concentration yields the equation at the right.

### Beer-Lambert Equation (solved for concentration)

$$c = A / (\varepsilon * b)$$

where:

A = UV absorbance in absorbance units (AU)

$\varepsilon$ = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) in liter/mol-cm

b = pathlength in cm

c = analyte concentration in moles/liter or molarity (M)

**Note**: Dividing the measured absorbance of a sample solution by its molar extinction coefficient yields the molar concentration of the sample. See Published Extinction Coefficients for more information regarding molar vs. mass concentration values.

## Extinction Coefficients for Proteins

The extinction coefficient of a peptide or protein is related to its tryptophan (W), tyrosin (Y) and cysteine (C) amino acid composition.

**Tip**: The extinction coefficient is wavelength specific for each protein and can be affected by buffer type, ionic strength and pH.

At 280 nm, the extinction coefficient is approximated by the weighted sum of the 280 nm molar extinction coefficients of the three constituent amino acids, as described in this equation:

$$\varepsilon = (nW * 5500) + (nY * 1490) + (nC * 125)$$

where:
$\varepsilon$ = molar extinction coefficient
n = number of each amino acid residue
5500, 1490 and 125 = amino acid molar absorptivities at 280 nm

## Available Options for Extinction Coefficient

This application offers six options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the $\varepsilon$ + MW (molar) or $\varepsilon$1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

**Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

- **1 Abs = 1 mg/mL**, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- **BSA** (Bovine Serum Albumin, 6.7 L/gm-cm)
- **IgG** (any mammalian antibody, 13.7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26.4 L/gm-cm)
- **Other protein** ($\varepsilon$ + MW), user-specified molar ext. coefficient
- **Other protein** ($\varepsilon$1%), user-specified mass ext. coefficient

**Note**: See Sample Type for details.

Most sources report extinction coefficients for proteins measured at or near 280 nm in phosphate or other physiologic buffer. These values provide sufficient accuracy for routine assessments of protein concentration.

## Published Extinction Coefficients

Published extinction coefficients for proteins may be reported as:

- wavelength-dependent molar absorptivity (or extinction) coefficient ($\varepsilon$) with units of $M^{-1}cm^{-1}$
- percent solution extinction coefficient ($\varepsilon$1%) with units of $(g/100\ mL)^{-1}cm^{-1}$ (i.e., 1% or 1 g/100 mL solution measured in a 1 cm cuvette)
- protein absorbance values for 0.1% (i.e., 1 mg/mL) solutions

**Tip**: Assess published values carefully to ensure unit of measure is applied correctly.

The equation at the right shows the relationship between molar extinction coefficient ($\varepsilon_{molar}$) and percent extinction coefficient ($\varepsilon$1%).

## Conversions Between $\varepsilon_{molar}$ and $\varepsilon$1%

$$(\varepsilon_{molar}) * 10 = (\varepsilon 1\%) * (MW_{protein})$$

Example: To determine percent solution extinction coefficient ($\varepsilon$1%) for a protein that has a molar extinction coefficient of 43,824 $M^{-1}cm^{-1}$ and a molecular weight (MW) of 66,400 daltons (Da), rearrange and solve the above equation as follows:

$$\varepsilon 1\% = (\varepsilon_{molar} * 10) / (MW_{protein})$$

$$\varepsilon 1\% = (43{,}824 * 10) / 66{,}400\ Da)$$

$$\varepsilon 1\% = 6.6\ g/100\ mL$$

To determine concentration (c) of a sample in mg/mL, use the equation at the right and a conversion factor of 10.

**Tip**: The NanoDrop Ultra software includes the conversion factor when reporting protein concentrations.

## Conversions Between g/100 mL and mg/mL

$$C_{protein}\ in\ mg/mL = (A / \varepsilon 1\%) * 10$$

Example: If measured absorbance for a protein sample at 280 nm relative to the reference is 5.8 A, protein concentration can be calculated as:

$$C_{protein} = (A / \varepsilon 1\%) * 10$$

$$C_{protein} = (5.8/6.6\ g/100\ mL) * 10$$

$$C_{protein} = 8.79\ mg/mL$$

Calculated protein concentrations are based on the absorbance value at 280 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction (or analysis correction) may be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm and 280 nm are used to calculate purity ratios for the measured protein samples.

Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

## Measured Values

### A280 absorbance

**Note**: For micro-volume absorbance measurements the spectra are normalized to a 10 mm pathlength equivalent.

- Protein absorbance values are measured at 280 nm using the normalized spectrum. If Baseline Correction is not selected, this is the reported A280 value and the value used to calculate protein concentration.
- If Baseline Correction is selected, the normalized and baseline-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

### A260 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at 260 nm is used to calculate A260/A280 ratios.

### Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **A260/A280 purity ratio**. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~0.57 is generally accepted as “pure” for proteins.

**Note**: Although purity ratios are important indicators of sample quality, the best indicator of protein quality is functionality in the downstream application of interest (e.g., real-time PCR).

- **Sample type**. Determines the extinction coefficient used in conjunction with Beer's Law to calculate sample concentration.
- **$R^2$ (280nm)**. Coefficient of determination for the line of best fit for 280 nm measurements.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays that the measurement was taken from the pedestal.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.
- **Mass extinction coefficient (1% solution)**.
- **Molar extinction coefficient**.
- **Molecular weight**.

8

# More Applications

Use the NanoDrop Ultra Spectrophotometers and Fluorometers to perform UV-Vis, OD600, Kinetics, or your own Custom measurements.

The UV-Vis application can be set up directly from the touchscreen and allows the instrument to function as a conventional spectrophotometer. Up to 40 wavelengths from 190 nm to 850 nm can be monitored and reported.

The Custom Methods application provides additional flexibility for users looking for unique information about their samples.

# UV-Vis

Measures the absorbance of any sample at up to 40 wavelengths across the ultra-violet (UV) and visible regions of the spectrum.

Measure UV-Vis

Reported Results

Settings

## Best practices for UV-Vis measurements

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to re-suspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near an analysis wavelength, you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Ensure samples are homogeneous before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

# Measure UV-Vis

The UV-Vis application allows the instrument to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 190 nm to 850 nm. Up to 40 wavelengths can be designated for absorbance monitoring and inclusion in the report. Automatic pathlength adjustment and a single-point baseline correction can also be used.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **More Apps** tab, then select **UV-Vis**.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
4. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

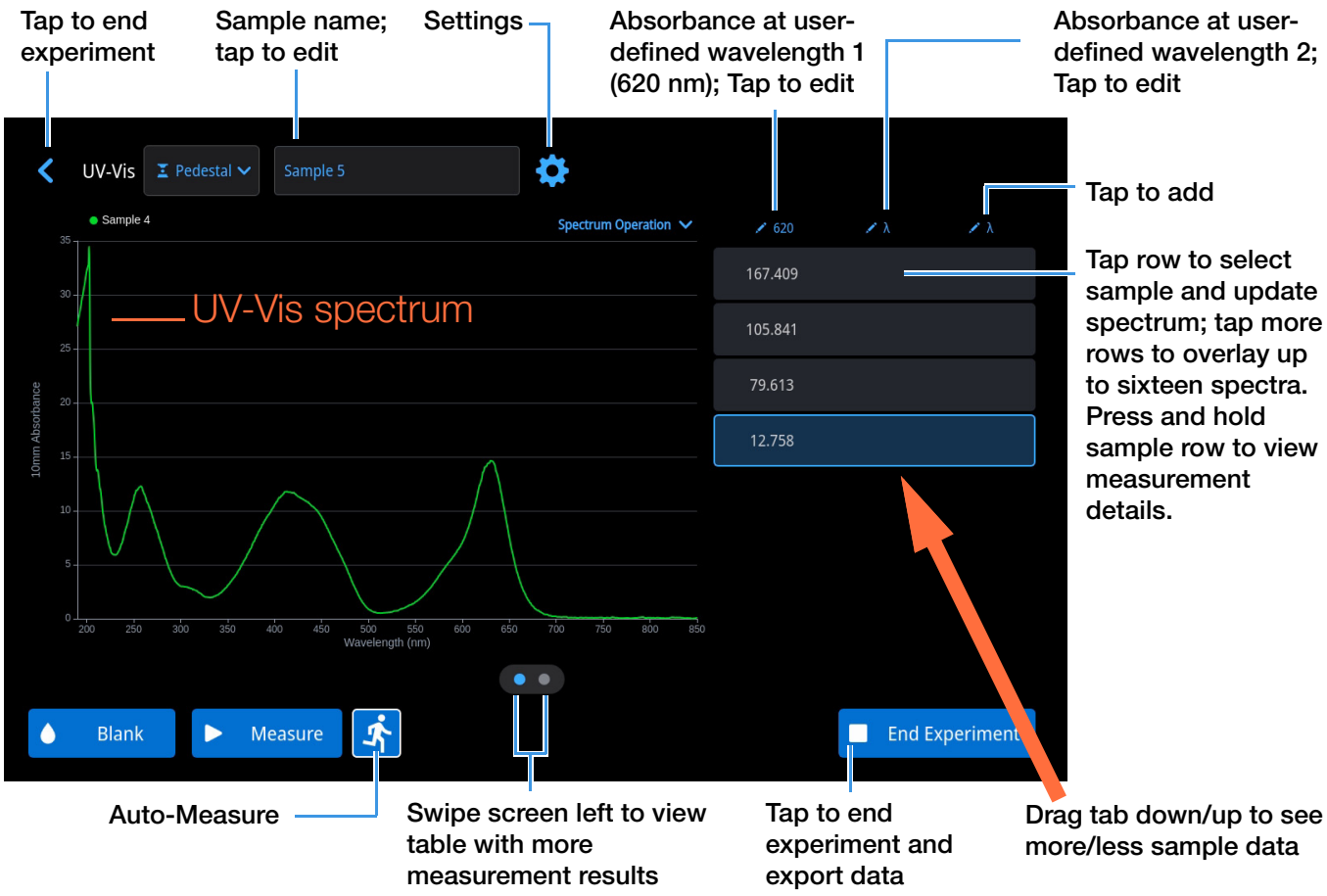
6. Lift the arm and clean both pedestals with a new laboratory wipe or remove the blanking cuvette.
7. Pipette 1-2 µL sample solution onto the pedestal, or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.
   - Cuvette: Select **Measure.**

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
9. When you are finished measuring samples, select **End Experiment**.
10. Lift the arm and clean both pedestals with a new wipe or remove the sample cuvette.

# Reported Results

## UV-Vis measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example as it appears on the NanoDrop Ultra local control instrument display:



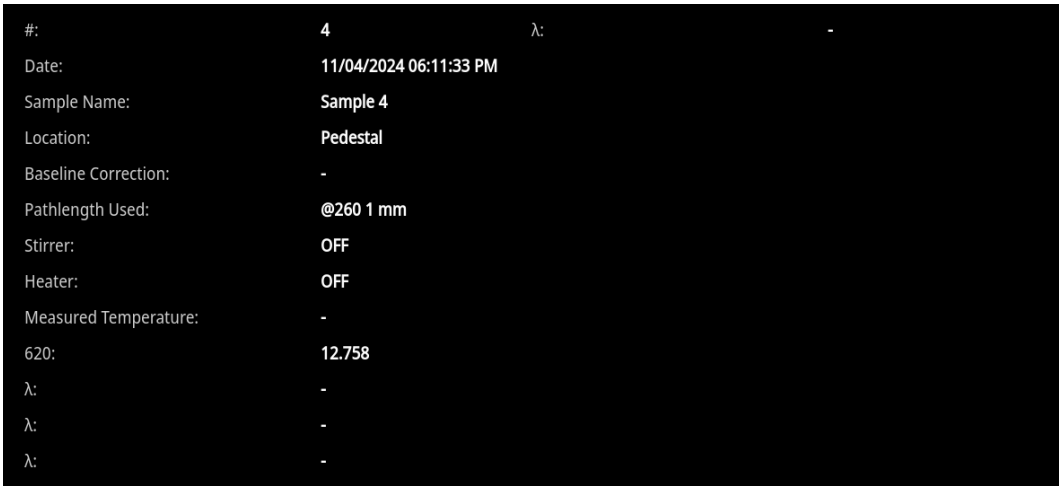

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## UV-Vis measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## UV-Vis reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



# Settings

The UV-Vis setup screen appears after you select the UV-Vis application from the More Apps tab on the home screen. To show the UV-Vis settings from the UV-Vis measurement screen, select  .

| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Monitored wavelengths | Enter up to 40 wavelengths between 190 nm and 850 nm | **User-defined wavelengths to be measured and reported at run time**. Absorbance values for the first three entered wavelengths are displayed in the measurement screen. To see absorbance values for 6 monitored wavelengths, swipe left in the measurement screen to show the Data table. To see all monitored wavelengths, press and hold a sample row to show the Sample Details screen (scroll up to display absorbance values for any additional user-defined wavelengths).<br><br>**Note**: If Baseline Correction is selected, all displayed absorbance values are the corrected values. |
| Analytical Wavelength | On or Off<br><br>Enter analytical wavelength in nm or use default value (220 nm) | This is the wavelength the software will use to determine the pathlength selection. Automated Pathlength cannot be used when Analytical Wavelength is enabled. |

| Setting | Available Options | Description |
|---|---|---|
| Automated Pathlength | On or Off<br>(affects pedestal measurements only) | **Optional automated pathlength selection**. Allows the software to use the optimal (shorter) pedestal pathlength for high concentration samples to help prevent detector saturation (see Detection Limits for details).<br>• When selected, the shorter pathlength is used when any wavelength between 220 nm and 850 nm has 10 mm equivalent absorbance value of 12.5 or higher. For wavelengths between 190 nm and 219 nm the change to the shorter pathlength occurs when any wavelength in this range has a 10 mm equivalent absorbance value of 10 or higher.<br>• When deselected, the pedestal pathlength is restricted to 10 mm across all wavelengths.<br>**Note**: In either case, displayed absorbance values have been normalized to a 10 mm pathlength equivalent. |
| Baseline Correction | On or off<br><br>Enter baseline correction wavelength in nm or use default value (750 nm) | **Optional user-defined baseline correction**. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

# Custom Methods

Create and run a custom measurement method to display unique information for your samples.



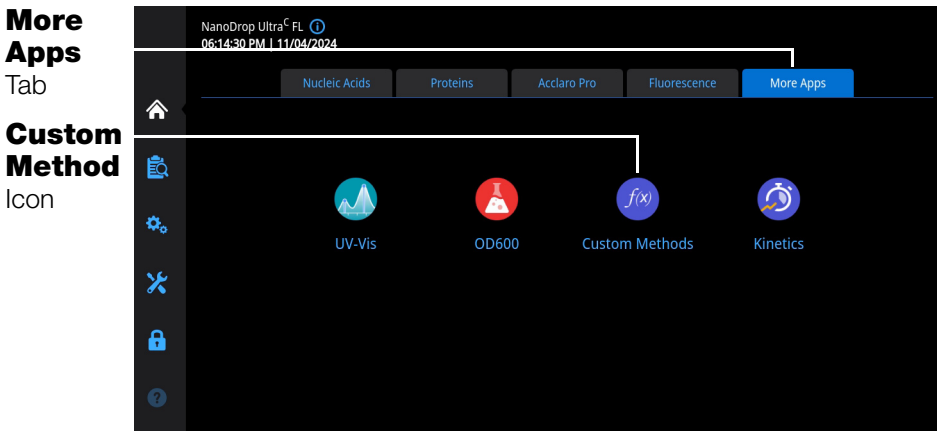
## Manage Custom Methods

Use the Custom Methods application to run a user-defined method created using the NanoDrop Ultra local or PC control software. Custom methods can be made with or without standards.

### Create custom method

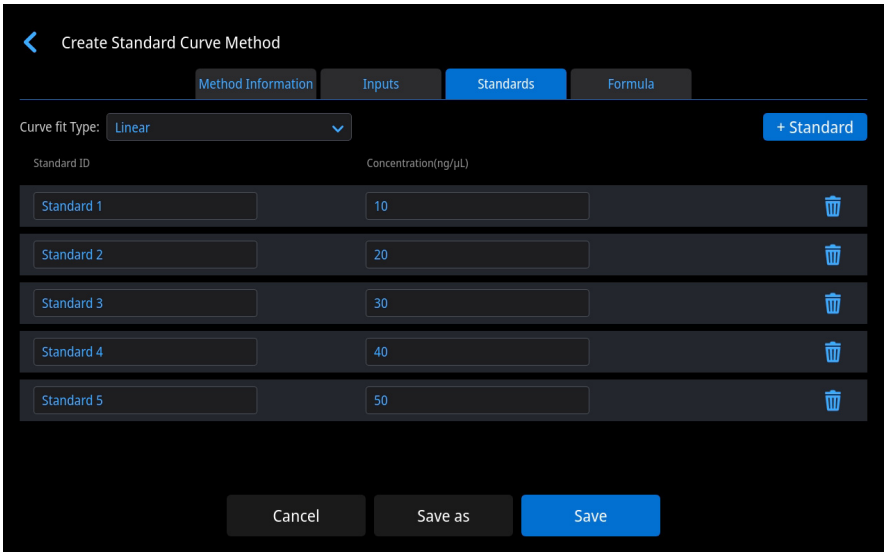
Create method to be used for sample measurements with user-defined settings.

1. From the home screen, select the **More Apps** tab, then select **Custom Methods**




2. Select + NEW METHOD and choose one of the following:
   - **Formula** (if your method will not have standards)
   - **Standard Curve** (if your method will have standards)

3. In the **Create Formula Method** window, enter **Method Name**.
4. Enter detailed **Description** of method, if desired.
5. Specify how to calculate and report the method results:
   - If method does not have standards, specify factor or extinction coefficient of analyte (enter "1" to report absorbance measurements only) within the **Inputs** tab.
   - If method has standards, enter name and concentration of each standard and select the curve fit type within the **Standards** tab.




   Alternatively, load a standard curve.
6. Enter or choose remaining custom settings as needed.
7. Choose **Save**

> **Note** Any errors in the method will be listed in orange text at the bottom of the method editor screen. Errors must be addressed before the method can be saved.

## View or edit custom method

1. From the Custom Methods screen, select ⋮ next to the desired method to edit.

2. Select **Edit**
3. View and adjust the method settings as desired
4. Select **Save**

## Copy custom method

To create a custom method that is similar to an existing one, open the existing method, make your changes, then select **Save As** and enter a new name.

### Copy custom method

1. From the Custom Methods screen, select  next to the desired method to copy.
2. Select **Edit**
3. View and adjust the method settings as desired.
4. Enter new **Method name** and **Description**.
5. Select **Save As**.
6. Enter a new filename for the method and click **Save**.

## Export custom method

Export a custom method in order to run it and store the measurement results on another NanoDrop Ultra instrument or another computer running the NanoDrop Ultra PC control software.

1. From the Custom Methods screen, select  next to the desired custom method.
2. Select **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported).
3. Select an available location to export to from the dropdown menu and then choose **Export** (method is exported to method file (*.method filename extension in proprietary format).

   To transfer the method to the NanoDrop Ultra instrument or NanoDrop PC control software, copy the method file to a USB memory device and then load the method (see Import a Custom Method for details)

## Import custom method

Import a custom method back to a computer running the NanoDrop Ultra software in order to edit the method settings.

1. From the Custom Methods screen, select **Import**

2. Locate and select ".method" file
3. Choose **Import** (imported method is added to end of Select Method list)

**Note** Custom methods downloaded from the NanoDrop website have a .zip file-name extension and must be extracted using a third-party file decompression program before the software will recognize them as custom methods.

### Delete custom method

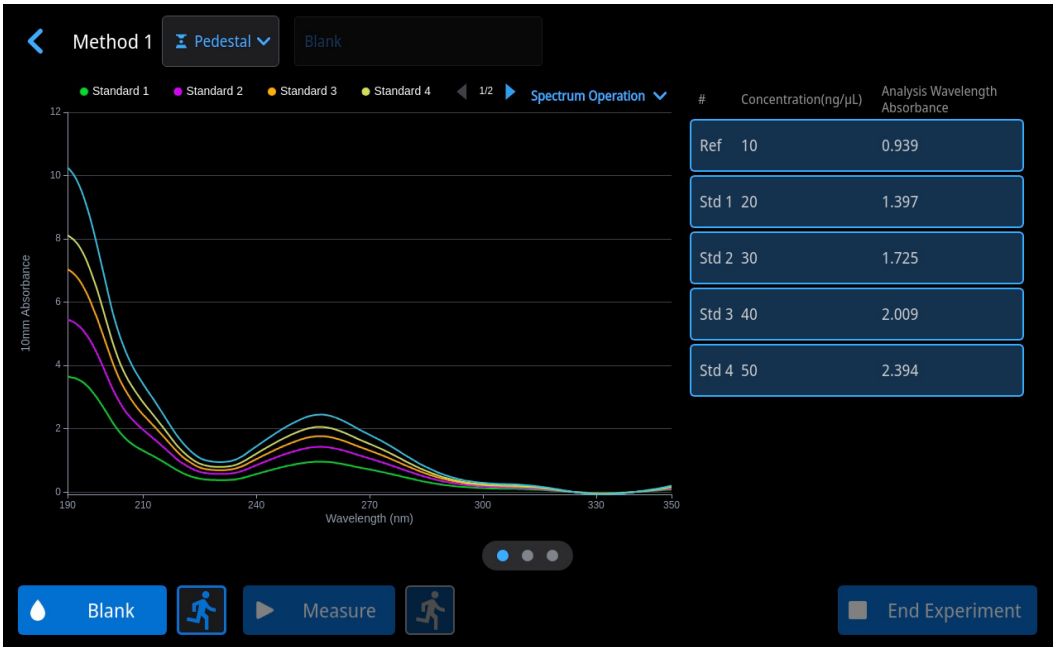
1. From the Custom Methods screen, select  next to the desired custom method.
2. Select **Delete**.
3. After the confirmation message, choose **Yes**.

### Load standard curve

1. From the Custom Methods screen, select **Load Standard**.

2.Select the desired previously run standard curve, then select **Load**.

The measurement screen opens with the standard curve loaded. You can begin by blanking and then measuring samples.




## Measure using a Custom Method

Use the Custom application to create and run a user-defined method created. For more information, see "Create custom method" on page 241.

### Before you begin...

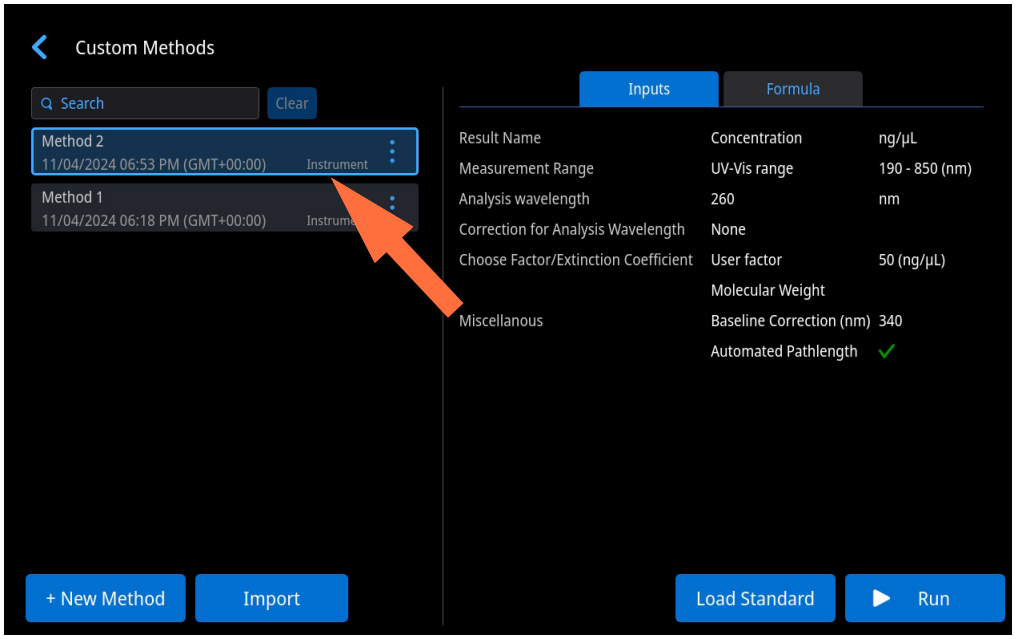
Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **More Apps** tab, then select **Custom Methods**.
2. In the method selection pane, select the method to run.



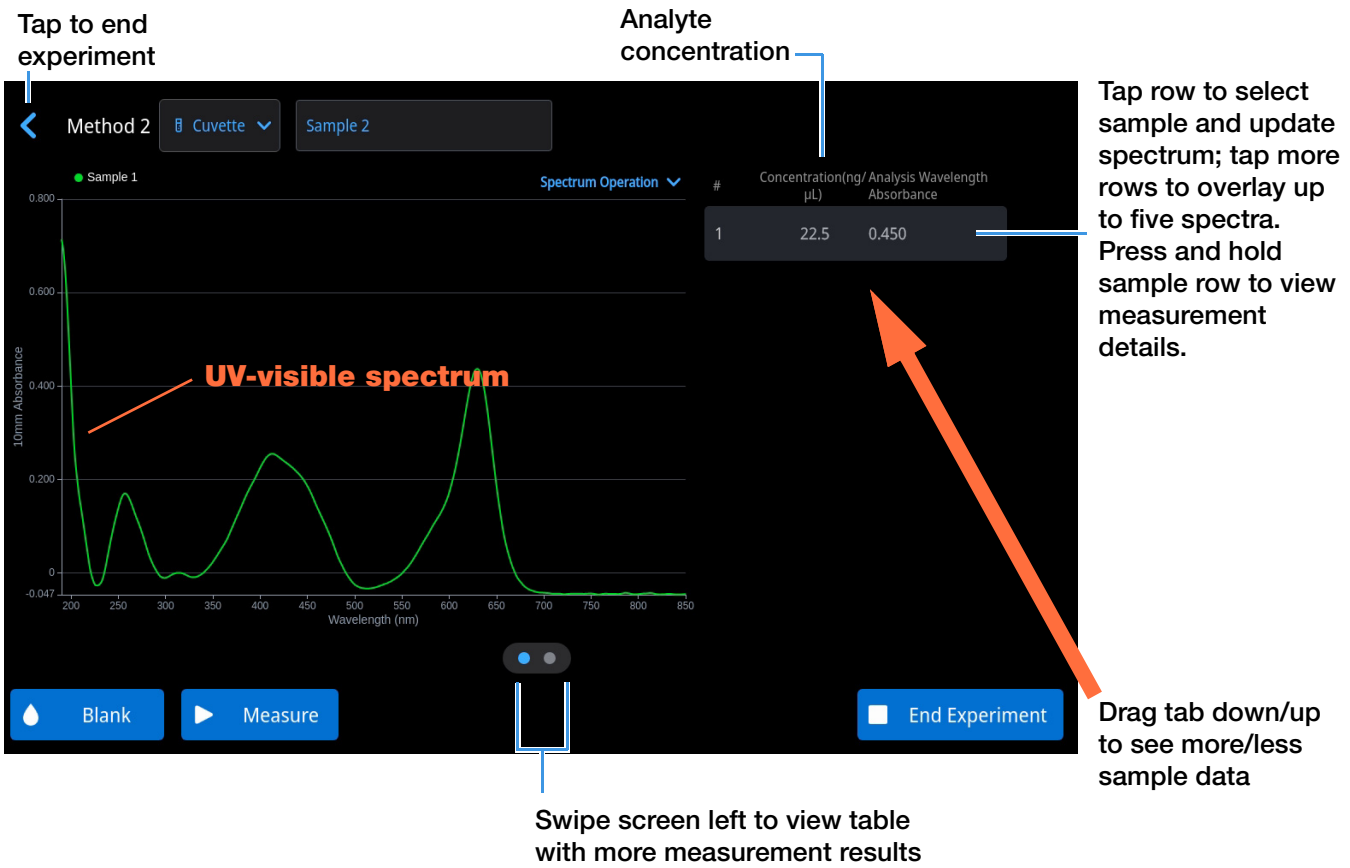

Information about the selected method appears in the Method Details pane.

3. Select Run.
4. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
5. Follow the on-screen instructions to measure a sample.

# Reported Results

## Custom method measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the NanoDrop Ultra local control software screen:



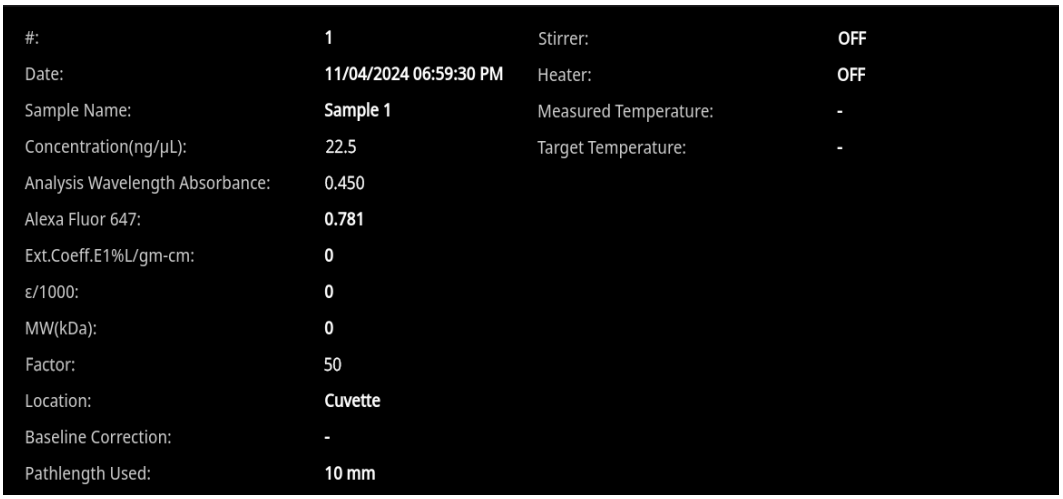

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## Custom method measurement screen (PC control)

For each measured sample and standard, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



| | | | |
|---|---|---|---|
| #: | 1 | Stirrer: | OFF |
| Date: | 11/04/2024 06:59:30 PM | Heater: | OFF |
| Sample Name: | Sample 1 | Measured Temperature: | - |
| Concentration(ng/µL): | 22.5 | Target Temperature: | - |
| Analysis Wavelength Absorbance: | 0.450 | | |
| Alexa Fluor 647: | 0.781 | | |
| Ext.Coeff.E1%L/gm-cm: | 0 | | |
| ε/1000: | 0 | | |
| MW(kDa): | 0 | | |
| Factor: | 50 | | |
| Location: | Cuvette | | |
| Baseline Correction: | - | | |
| Pathlength Used: | 10 mm | | |

# Settings

These settings are available for creating custom methods.

| Setting | Available Options |
|---|---|
| Result | Enter descriptive name for calculated concentration result (for example, “MTT Assay”) and use adjacent drop down list to select appropriate unit. Result name appears as column heading for reported concentration value. |
| Measurement range | Select spectral range in which method will acquire data.<br>Available options:<br>• Ultra-violet only (190 nm - 350 nm)<br>• Visible only (350 nm - 850 nm)<br>• Ultra-violet and visible (190 nm - 850 nm)<br>• Custom (specify starting and ending point in nanometers)<br>**Notes**:<br>• If a Baseline correction and/or Analysis wavelength correction are used, make sure your selected spectral range includes your specified baseline correction and/or analysis correction wavelength.<br>• For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent. |
| Analysis wavelength correction | Use this option to specify absorbance correction at analysis wavelength only.<br>Available options:<br>• **None.** No correction at analysis wavelength.<br>• **Single point**. Enter wavelength for analysis correction. (Absorbance value at specified analysis correction wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.)<br>• **Sloping baseline**. Enter two wavelengths that define sloping baseline for analysis correction. (Absorbance value of sloping baseline at analysis wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.) |

| Setting | Available Options |
|---|---|
| Factor or Extinction coefficient at 1 cm pathlength (Formula methods only) | Specify whether to use factor or extinction coefficient to calculate concentration result:<br>• **User-defined factor**. Enter **factor** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how factor is used to calculate sample concentration:<br>$c = (A * f) / b$<br>where:<br>c = analyte concentration<br>A = absorbance in absorbance units (A)<br>**f** = factor (typically 1/ε, where ε = wavelength-dependent molar absorptivity coefficient, or extinction coefficient)<br>b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent)<br>• **Extinction coefficient and molecular weight**. Enter **extinction coefficient** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how extinction coefficient is used to calculate sample concentration:<br>$c = A / (\varepsilon * b)$<br>where:<br>c = analyte concentration<br>A = absorbance in absorbance units (A)<br>ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient)<br>b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent) |
| | **Notes**:<br>• Refer to product literature for information about factors and extinction coefficients for specific materials.<br>• To set up a method that reports absorbance measurements only, select Factor or Extinction Coefficient with the factor or extinction coefficient set to “1”.<br>• If specified unit for factor or extinction coefficient is based on mass (such as mg/mL) and specified unit for calculated result is based on molarity (such as pmol/µL) or vice versa, enter **molecular weight** and use adjacent drop down list to select appropriate **unit**. |

| Setting | Available Options |
|---|---|
| Standards (Standard curve methods only) | Define the standards:<br>• Enter name and analyte concentration of each standard and a reference, if desired:<br>– Depending on the Curve Type setting, a standard curve can be generated using two or more standards. (The software **allows a reference and up to 15 standards**.)<br>– **All reference and standards solutions** should be in the same buffer used to resuspend the samples plus the same volume of reagent added to the samples.<br>– **First standard** can be a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement.)<br>– **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest.<br>– **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard. |

| Setting | Available Options |
|---|---|
| | • Select curve fit type.<br><br>Specify type of equation used to create standard curve from standard concentration values. Available options:<br><br>– Linear: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard). Linear through zero is similar but with the constraint that the line passes through the origin (0,0)<br><br>– Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement ans at least one standard)<br><br>– $2^{nd}$ order polynomial: Draws the $2^{nd}$ order least squares polynomial using all measured standards (requires reference measurement and at least standards). $2^{nd}$ order polynomial through zero is similar but with the constraint that the line passes through the origin (0,0)<br><br>– $3^{rd}$ order polynomial: Draws the $3^{rd}$ order least squares polynomial using all measured standards (requires reference measurement and at least three standards). $3^{rd}$ order polynomial through zero is similar but with the constraint that the line passes through the origin (0,0) |
| Analysis wavelength (Standard curve methods only) | Monitor absorbance at specified wavelength (enter the wavelength in nanometers).<br><br>**Note**: The specified wavelength must fall within the selected measurement range.<br><br>The measurement results or the concentration will be calculated automatically using the absorbance value at the specified wavelength and applying the selected method type (factor or standard curve). |
| Baseline correction | Select this option to correct offset caused by light scattering particulates by subtracting the absorbance at a specified baseline point. Then specify wavelength for baseline correction.<br><br>**Note**: Software subtracts absorbance value at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. |

| Setting | Available Options |
|---|---|
| Automated pathlength | Affects micro-volume measurements only.<br>• When Automated Pathlength is selected, software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength. For example, when sample absorbance at the analysis wavelength is less than or equal to 12.5 (10 mm pathlength equivalent), the optimal longer pathlength is used. When sample absorbance is greater than 12.5, the optimal shorter pathlength is used. Recommended for samples that are highly absorbing at the analysis wavelength. (This option may cause reduced sensitivity when the sample spectra have a large absorbance peak that is not at the analysis wavelength.)<br>**Note**: When the analysis wavelength is between 190 nm and 219 nm, the optimal longer pathlength is used when sample absorbance is less than or equal to 10 (10 mm pathlength equivalent), and the optimal shorter pathlength is used when sample absorbance is greater than 10.<br>• When Automated Pathlength is deselected, the software uses a 1 mm pathlength regardless of the sample absorbance. This can cause detector saturation (resulting in jagged peaks) for highly absorbing samples (e.g., ~15 A at 10 mm pathlength equivalent). |

| Setting | Available Options |
|---|---|
| Formula table (optional) | Use the Formula table to specify additional reported results, such as a purity ratio, for each sample.<br><br>Available options:<br><br>• **Predefined**. Select from a list of predefined formulas, which can be used as is or edited, and choose **Save**. The predefined formula is listed in the Formula Table.<br><br>• **+Formula**. Create formula for current method. Available options:<br><br>  • **Formula Name**. Enter a name for the formula. After a measurement, the name is reported in Data Table and Sample Details screens.<br><br>  • **Equation**. Enter valid formula (see below for rules and examples). After a measurement, the measured or calculated value is reported in Data Table and Sample Details screens.<br><br>  • **Unit**. Select from a drop-down of available units for reported result. After a measurement, the unit is reported in Data Table and Sample Details screens.<br><br>• **Delete**. Delete selected formula from current method by selecting  . |
| Formula rules | Custom formulas can include the following operators and functions:<br><br>• **Path()**. Returns sample pathlength in cm.<br><br>• **A(nm)**. Returns sample absorbance at specified wavelength (for example, enter A(650) to add the measured absorbance at 650 nm to your equation).<br><br>• **Operators**: + (add), - (subtract), * (multiply), / (divide).<br><br>• **Functions**: Log(x), Pow(x,y).<br><br>**Notes**: Follow these additional rules for all languages:<br><br>• Use period “.” decimal separators for floating point and double-floating point numbers.<br><br>• Use comma “,” list separators (for example, “POW(2,8)”).<br><br>• Do not use comma “,” group separators for large numbers (for example, enter 1000 rather than 1,000). |

# OD600

Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.

Measure OD600

Reported Results

Settings

Calculations

## Theory of OD600 application

The OD600 application measures light transmission and uses that value to calculate absorbance. In spectroscopy, transmitted light is defined as any light that is not absorbed by, reflected from and scattered off a sample.

In the case of living cells, most of the incident light is transmitted through the sample rather than scattered, reflected or absorbed. The amount of scattered light is low and can vary from instrument to instrument. As a result, calculated absorbance readings are typically very low.

The calculated absorbance values are used to determine the density of cells in solution in cells/mL. The physical concepts and formulas that relate optical properties of living cells to concentration include:

- Cells, which have a different index of refraction from the surrounding medium, randomly reflect and scatter light out of the incident light path. The amount of scattering is proportional to the density of cells in the sample.
- The Beer's Law equation is used to relate absorbance to concentration. See Calculations for OD600 Measurements for details.
- For cuvette reading with the NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL instruments, accurate absorbance readings are typically in the range between 0.04 A and 1.5 A. Serial dilutions of the sample are usually needed to bring the absorbance readings within this range.
- All measurements should be made on the same type of spectrophotometer and method (i.e., pedestal vs. cuvette) as the amount of scattered light captured varies based on the optical configuration. When using a different spectrophotometer or method, calculate and apply a conversion factor to the reported results. For example, to compare OD readings using the pedestal vs. a cuvette, a conversion factor can be calculated as follows:

  Conversion factor = Cuvette OD/Pedestal OD

## Best practices for OD600 measurements

- Ensure the sample is within the instrument's absorbance detection limits.
- Blank with the growth or culture media the cells of interest are suspended in.
- Run a blanking cycle to assess the absorbance contribution of your media solution. If the media solution exhibits strong absorbance at or near the analysis wavelength (600 nm), you may need to choose a different media solution or application. See Choosing and Measuring a Blank for more information.
- Make dilutions as necessary to ensure sample cultures do not exceed the linear dynamic range of the assay before the culture reaches the stationary phase. The linear range depends largely on optical configuration and, therefore, differs for pedestal and cuvette measurements. To determine the linear range:
  - Measure a series of dilutions using a young overnight culture (~16 hrs) of the microbial strain
  - Graph the OD600 measurements against the dilution factor

  The upper detection limit is the measured OD600 value at which there ceases to be a linear correlation between dilution factors and OD600 readings.
- Mix samples gently but thoroughly immediately before taking an aliquot for measurement.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Avoid introducing bubbles when mixing and pipetting.
  - Start the measurement promptly to avoid settling or evaporation.
  - Follow best practices for micro-volume measurements.
  - Use 2 µL sample volume. See Recommended Sample Volumes for more information.
  - For dilute samples that exhibit low absorbance at 600 nm, use an alternative wavelength such as 400 nm to measure absorbance, or use cuvettes instead of micro-volume measurements.
- For cuvette measurements (NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL instruments only):
  - Use clean plastic, glass or quartz cuvettes.
  - Follow best practices for cuvette measurements.
  - Do not use the automatic stirring feature for this assay.

# Measure OD600

Use the OD600 application to monitor the growth rate of bacterial or other microbial cell cultures by measuring the optical density (absorbance) of the culture in growth media at 600 nm. The Beer-Lambert equation and a user-entered conversion factor are used to correlate absorbance with concentration. Reported concentration values can be used to identify the phase of cultured cell populations, e.g., log or exponential and stationary.

The OD600 application reports cell concentration in cells/mL. A single-point absorbance correction can be used. This application does not require a standard curve.

### Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

**Note** Due to the amount of scattered light present in this assay, absorbance readings are typically very low.

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

## Procedure

1. From the home screen, select the **More Apps** tab, then select **OD600**.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.

4. Pipette 2 µL blanking solution (i.e., the media solution the cells of interest are suspended in) onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   **Tip**: If using a cuvette, make sure to align the cuvette light path with the instrument light path.

5. Select **Blank** and wait for the measurement to complete.

   **Tip**: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.

7. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.

8. Start the sample measurement:

   – Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   – Cuvette: select **Measure**

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.

10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

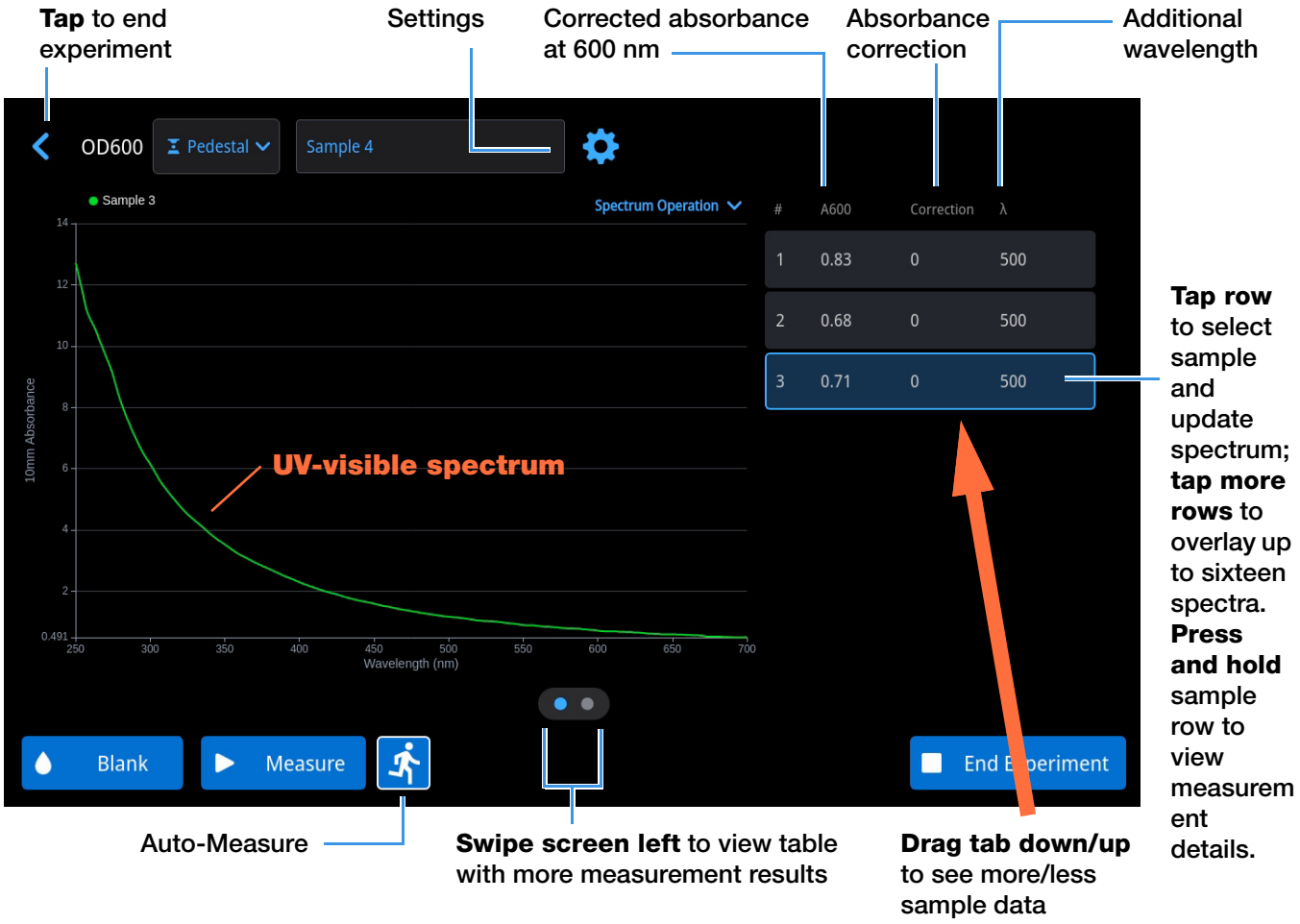
## Related Topics

- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## OD600 measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the NanoDrop Ultra local control software:



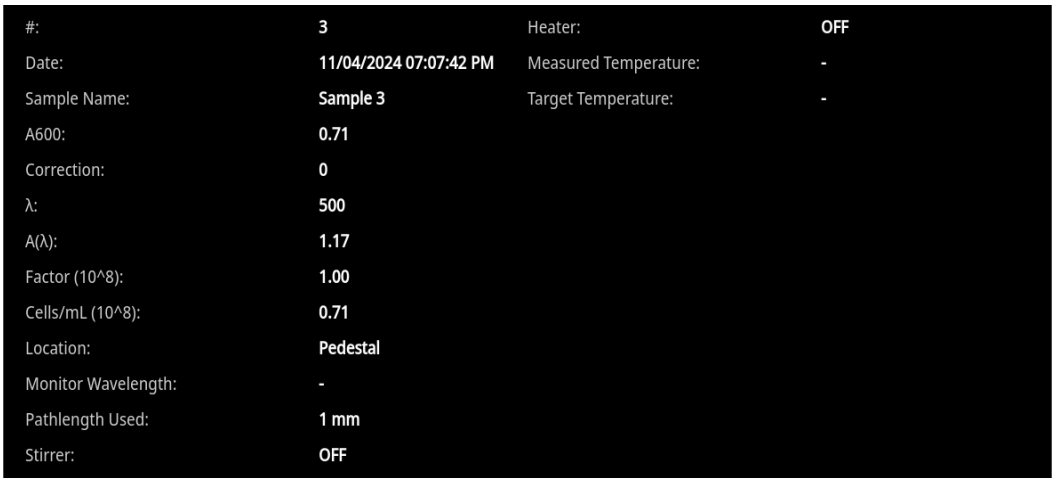

**Note** Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

## OD600 measurement screen (PC control)

For each measured sample and standard, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

## Reported values

The initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

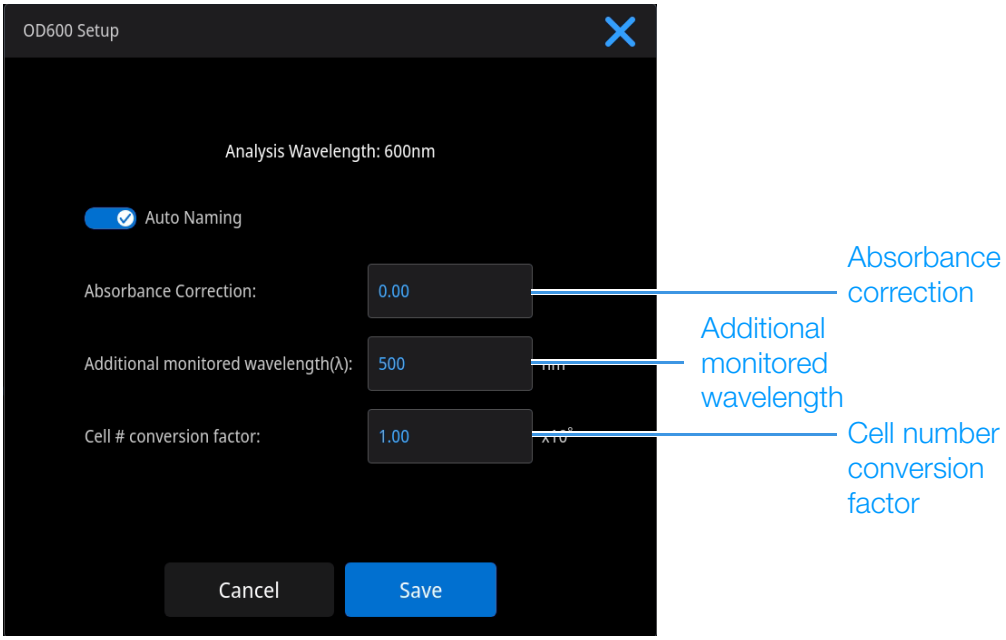



## Related Topics

- Basic Instrument Operations
- OD600 Calculations

# Settings

The OD600 setup screen appears after you select the OD600 application from the More Apps tab on the home screen. To show the OD600 settings from the OD600 measurement screen, select .




| Setting | Available Options | Description |
|---|---|---|
| Auto Naming | On or off | When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name. |
| Absorbance correction | Absorbance value between 0 and 300 A | User-defined absorbance correction. Enter absorbance correction for displayed spectrum. This can be useful, for example, to correct baseline offset caused by any difference between the media solution used to blank the instrument and media used to suspend the cell culture sample, and because scattered light generally produces an offset.<br><br>Absorbance correction value is subtracted from absorbance values at all wavelengths in sample spectrum. (All displayed absorbance values are corrected values.) |

| Setting | Available Options | Description |
|---|---|---|
| Additional monitored wavelength (λ) | Any wavelength between 250 nm and 700 nm | User-defined wavelength. Enter an additional wavelength to measure if desired (useful for dilute samples that exhibit low absorbance at 600 nm).<br><br>If an alternative wavelength is specified, use this equation to calculate cell concentration:<br><br>$c = A(\lambda) * factor(\lambda)$<br><br>where:<br><br>c = analyte concentration in cells/mL<br><br>A(λ) = UV-visible absorbance at specified wavelength in absorbance units (A)<br><br>factor(λ) = 1/(ε(λ) * b) in mL/cell-cm<br><br>where:<br><br>ε(λ) = molar absorption coefficient (or extinction coefficient) at specified wavelength<br><br>b = pathlength in cm (1.0 cm for the NanoDrop Ultra instruments) |
| Cell number conversion factor ($10^8$) | Any number | User-defined factor. Generally accepted factor for measured cell type, or one derived empirically using a solution of study cells at known concentration using the same media.<br><br>Default value is $1x10^8$ which is the generally accepted factor for most bacterial cell suspensions such as E. coli.<br><br>**Tip**: The factor is wavelength specific for each cell type and can be affected by the type of media used for the measurements. Ideally, the factor should be determined empirically using a solution of the study cells at a known concentration using the same media. |

## Related Topics

- Instrument Settings

# Calculations

Similar to the nucleic acid applications, the OD600 application uses a modification of the Beer-Lambert equation to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

The OD600 application offers a user-specified factor, to be used in conjunction with Beer's Law to calculate sample concentration. If the factor is known, enter the factor. Otherwise, use $1x10^8$, which is the generally accepted factor for most bacterial cell suspensions such as E. coli.

Calculated cell concentrations are based on the absorbance value at 600 nm, the entered factor and the sample pathlength. A single-point absorbance correction may be applied.

### Measured Values

#### A600 absorbance

**Note**: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Cell culture absorbance values are measured at 600 nm using the normalized spectrum. If no Absorbance Correction is specified, this is the reported A600 value and the value used to calculate cell concentration.
- If an Absorbance Correction is specified, the normalized and (absorbance) corrected absorbance value at 600 nm is reported and used to calculate cell concentration.

#### A(λ) absorbance

- Normalized and (absorbance) corrected (if used) absorbance value at any specified Additional Monitored Wavelength (λ) is also reported.

### Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized. (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

### Reported Values

**Cell concentration.** Reported in cells/mL. Calculations are based on Beer-Lambert equation using corrected A600 absorbance value.

# Kinetics

Make time-based kinetic measurements using the cuvette holder (NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL model instruments only).

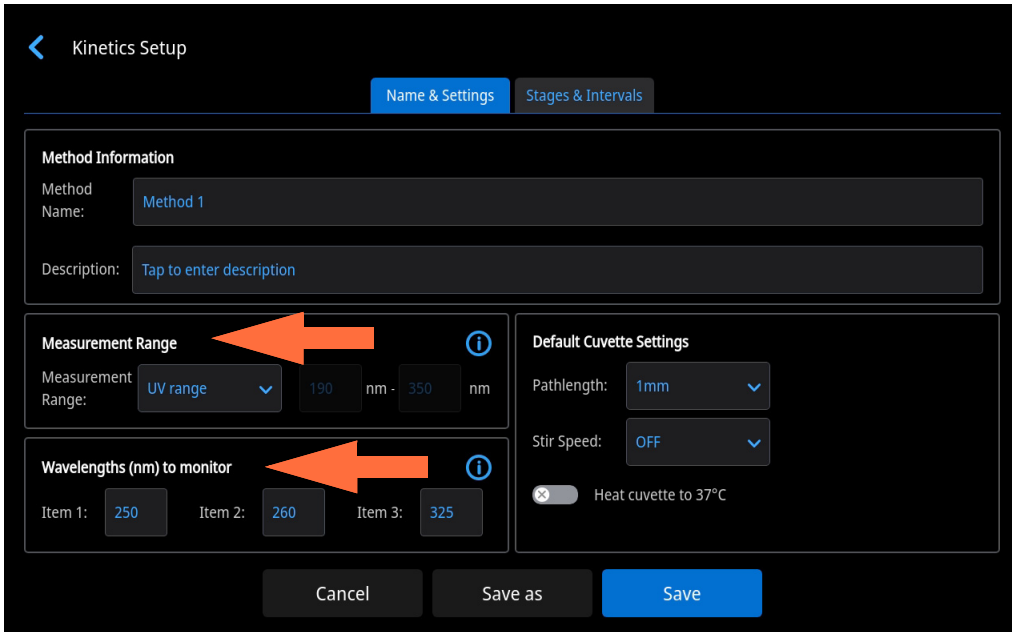


## Manage Kinetics Methods

### Create kinetics method

1. From the home screen, select the **More Apps** tab, then select **Kinetics**.
2. Select **+ New Method**.
3. Enter **Method Name** and **Description** (if desired), select **Measurement range** (use the drop down to select ranges, shown below) and specify up to three **Wavelengths to monitor.**

   Select all applicable cuvette specific settings (pathlenth, stir speed, and heating)



4. Select the Stages and Intervals tab. The stages and intervals settings are displayed.


5. Select **Number of stages** and **Time unit** (minutes or seconds)
6. For each stage, specify **# intervals**, **Interval times** and any **Delays** between stages

   The colored rows and boxes at the right visually represent the specified stages. The colored **rows** show the start and end times for each stage; the colored **boxes** correspond with the specified delay and number of intervals for each stage.

7. To save the method and return to the Kinetics menu, select **Save**.

**Note** The **Method Name**, **Interval Time**, and **# Intervals** must be defined before you are able to save the method.

## View or edit kinetics method

1. From the Kinetics screen, select ⋮ next to the desired method to edit.
2. Select **Edit**.
3. View and adjust the method settings as desired.
4. Select **Save**.

## Copy kinetics method

To create a kinetics method that is similar to an existing one, open the existing method, make your changes, then select Save As and enter a new name.

1. From the Kinetics screen, select  next to the desired method to copy.
2. Select **Edit**.
3. View and adjust the method settings as desired.
4. Enter new **Method Name** and **Description**.
5. Select **Save As**.
6. Enter a new name for the method and click **Save**.

## Export kinetics method

Export a kinetics method in order to run it and store the measurement results on another NanoDrop Ultra instrument or another computer running the NanoDrop Ultra PC control software.

1. From the Kinetics screen, select  next to the desired method to export.
2. Select **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported).
3. Select an available export location from the drop-down menu and choose **Export** (method is exported to method file (*.method filename extension) in proprietary format).

To transfer the method to another NanoDrop Ultra instrument or NanoDrop Ultra PC control software, copy the method file to a USB memory device and then load the method (see Import kinetics method for details).

## Import kinetics method

Import a kinetics method back to a NanoDrop Ultra instrument or a computer running the NanoDrop Ultra PC control software to run or edit the method.

1. From the **Kinetics** screen, select **Import**.
2. Locate and select ".method" file.
3. Select **Import** (imported method is added to end of the method selection pane).

## Delete kinetics method

1. From the **Kinetics** screen, select  next to the desired method to delete.
2. Select **Delete**.
3. After the confirmation message, select **Yes**.

# Measure Kinetics

The NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL model instruments can be used to make time-based kinetic measurements on samples in cuvettes. Up to 3 wavelengths between 190 nm and 850 nm can be designated for continuous absorbance monitoring at user-defined intervals in up to 5 stages. Cuvette measurements offer an extended lower detection limit and an optional 37 °C heater and micro-stirrer.

**Note** The instrument arm can be up during cuvette measurements, which allows you to add reagents to the sample solution if desired.

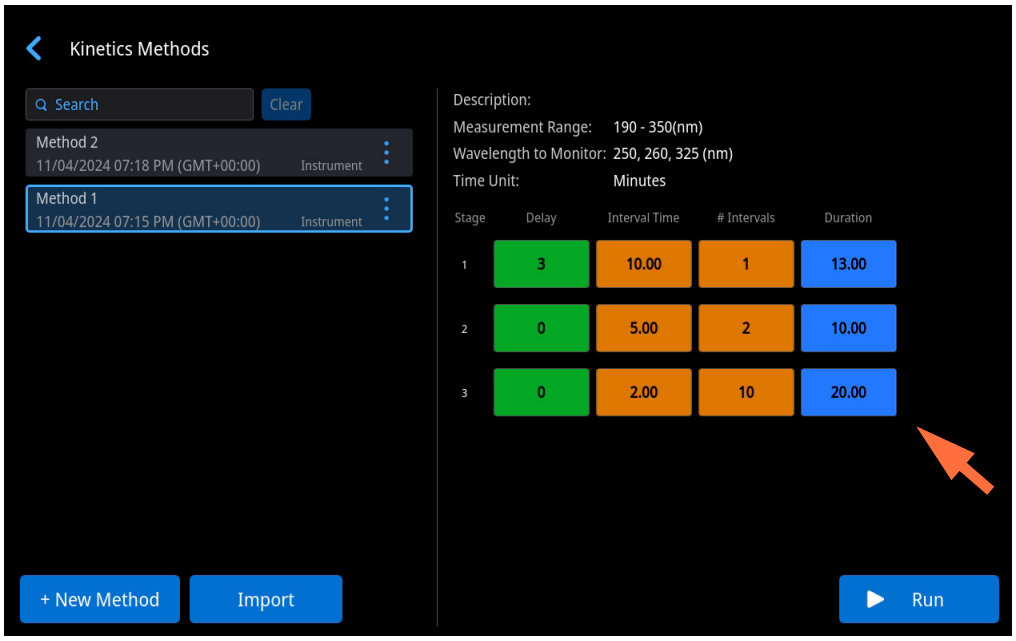
## To make kinetic measurements

**NOTICE**

- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.

## Procedure

1. From the home screen, select the **More Apps** tab, then select **Kinetics**.
2. In the method selection pane, select the method to run.




Information about the selected method appears in the method details pane.

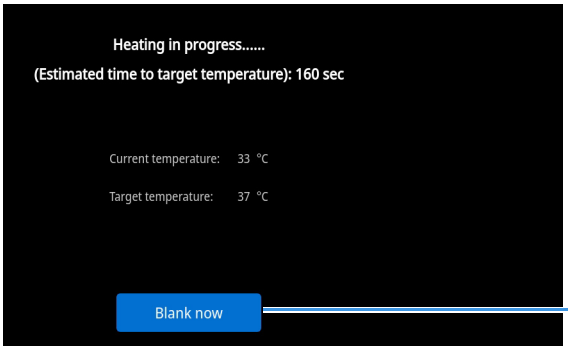
3. Select 

.

   **Note**: If your cuvette pathlength is not 10 mm, specify the correct pathlength in Kinetics Settings.

4. Measure a blank:
   - Fill clean, dry cuvette with enough blanking solution to cover instrument optical path
   - Lift instrument arm and insert blanking cuvette into cuvette holder, making sure to align light path of cuvette with light path of instrument
   - Select **Blank**

     If **Heat Cuvette to 37 °C** is selected in see Kinetics Settings, a message tells you the current temperature and waits for the heater to reach the target temperature before starting the measurements:




**To override the wait and start the blank measurement immediately, tap Blank Now.**

   - Wait for blank measurement to complete and then remove cuvette

     **Note**: The heater target temperature is not adjustable.

5. Measure a sample:
   - Fill clean, dry cuvette with enough sample solution to cover optical path
   - Insert sample cuvette into cuvette holder, making sure to align light paths
   - Select **Measure**

     If **Heat Cuvette to 37 °C** is selected in Kinetics Settings, a message tells you the current temperature and waits for the heater to reach the target temperature before starting the measurements:

     **Note**: You may add reagents to the sample solution at any time during the measurement

     Use the **Pause and Resume** button at the bottom of the measurement screen to pause and resume the experiment (if you need to end the experiment early, select **Stop**)

- Wait for all measurement stages to complete
- Remove cuvette and clean it according to manufacturer specifications

Results for each measurement in each interval are displayed in real time. When all stages are completed, the spectra and reported values for the entire experiment are displayed.

6. When you are finished reviewing the data, select **End Experiment**. Each saved experiment contains one complete set of kinetic measurements based on the selected method.

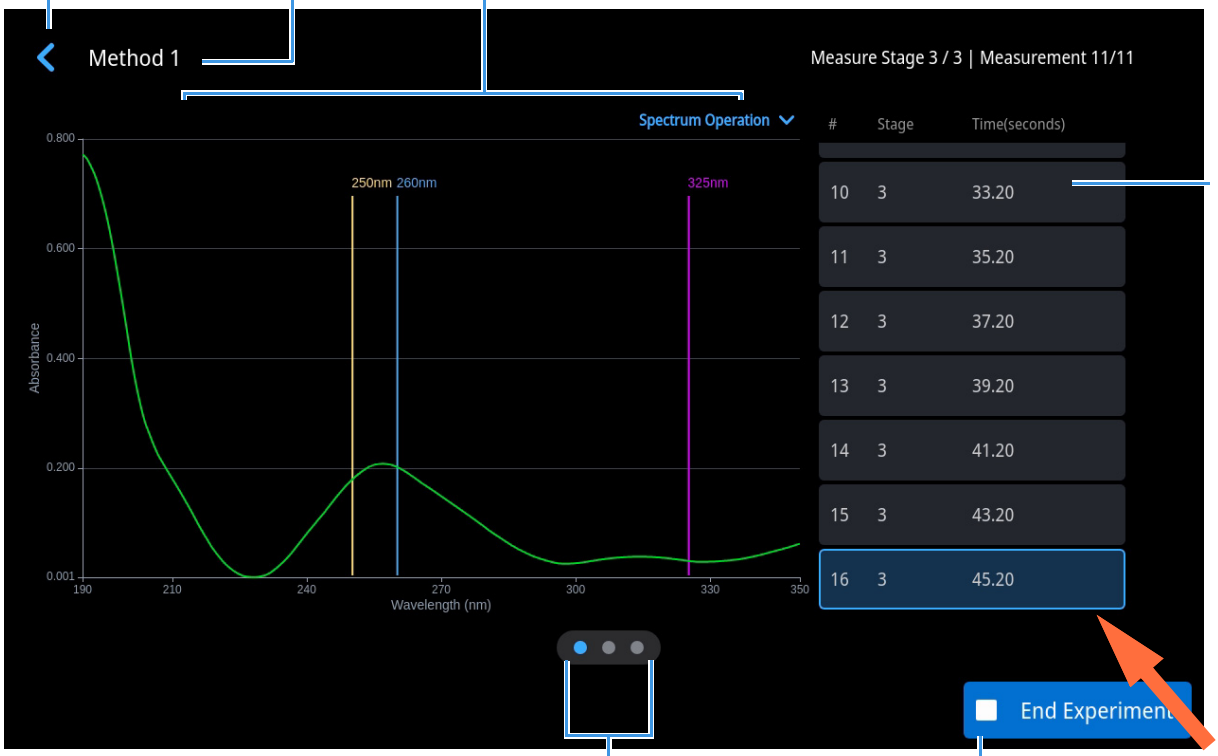
## Related Topics

- Measure a Sample Using a Cuvette
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

# Reported Results

## Absorbance measurement screen (local control)

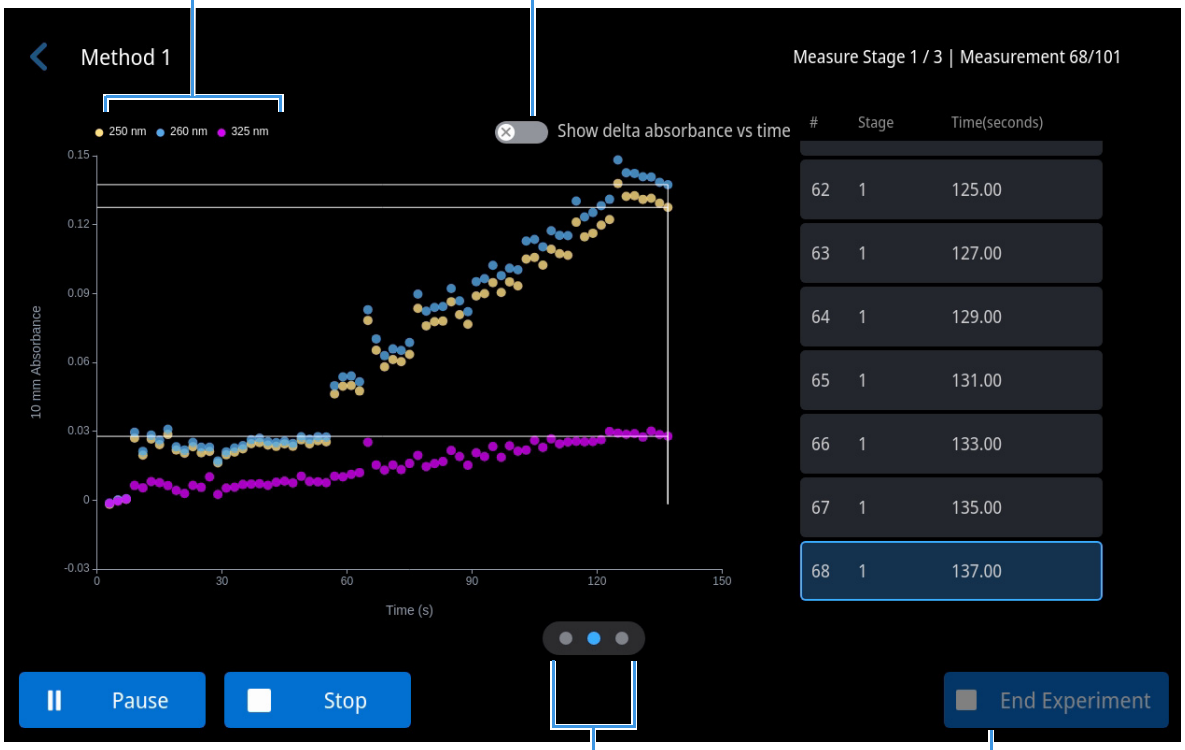
The absorbance measurement screen appears immediately after you select **Measure** in the kinetics experiment. This screen shows the absorbance spectrum for each measurement, with wavelength on the X-axis and absorbance on the Y-axis. Vertical lines indicate the specified wavelengths to monitor. The list at the right shows the time each measurement was taken in each specified stage (drag the tab down and up to see more entries). Each item in the list at the right has a corresponding absorbance spectrum at the left. The following image highlights the available features on the NanoDrop Ultra local control software.



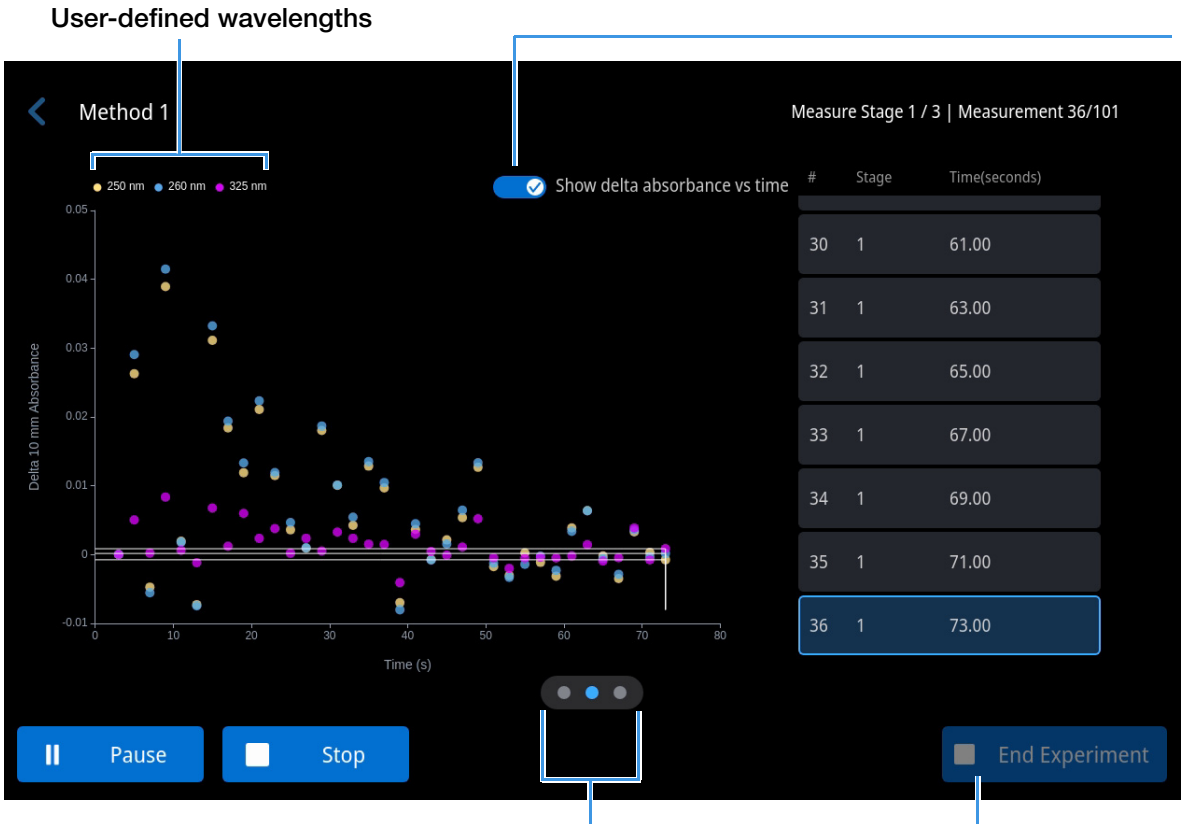

**Note** For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

## Rate measurement screen (local control)

To see the Rate measurement screen, swipe the absorbance measurement screen (see above) to the left. The Rate measurement screen shows a sample's absorbance measured at each user-defined wavelength over time, with time on the X-axis and absorbance on the Y-axis. Measurements taken at each specified wavelength are presented in a unique color. A key showing the monitored wavelengths and their assigned colors appears in the upper left corner of the screen.

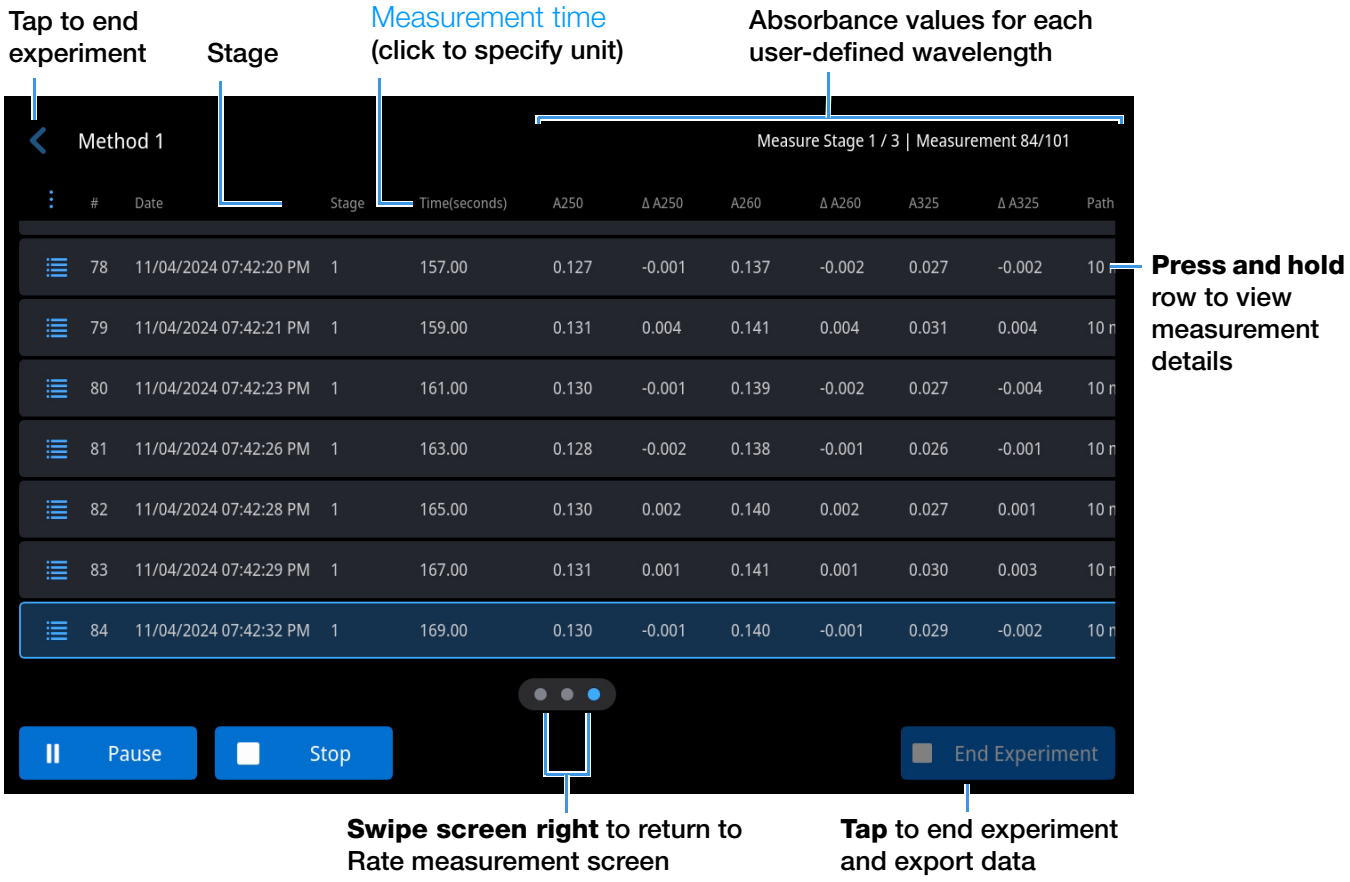


Use the slider button next to **Show Delta Absorbance Vs Time** to show the change in measured absorbance over time, where each data point is the difference in absorbance from the previous measurement.



## Data Table (local control)

To see the data table, in local control, swipe the rate measurement screen to the left. Each row in the table shows the absorbance values at all user-defined wavelengths at a given stage and time. Scroll down to see measurement information that is out of view. The image below highlights the available features.

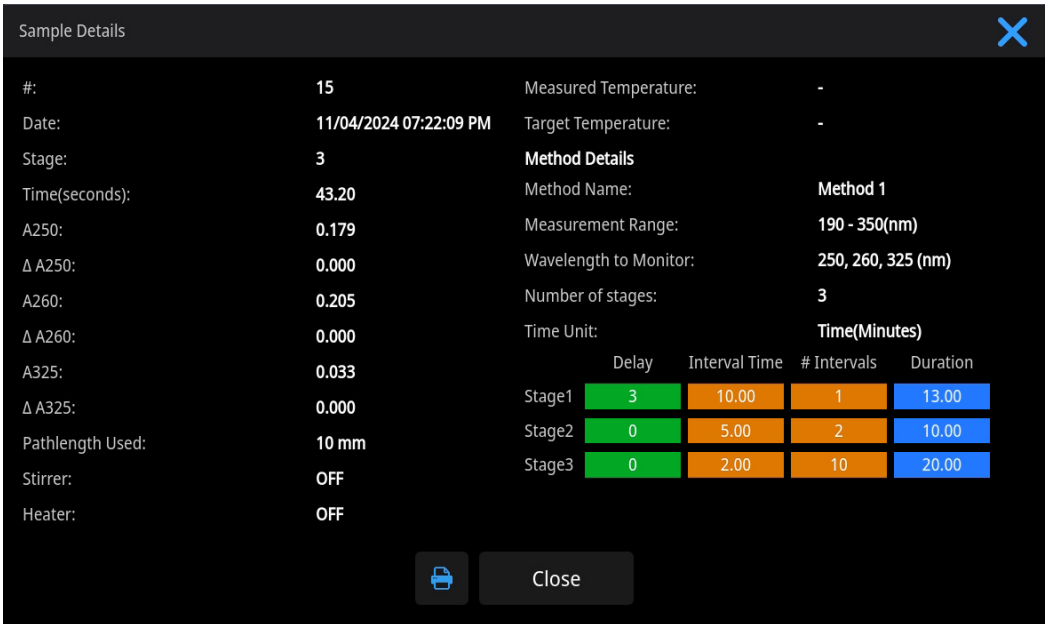



## Absorbance measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

## Reported values

The initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:




## Related Topics

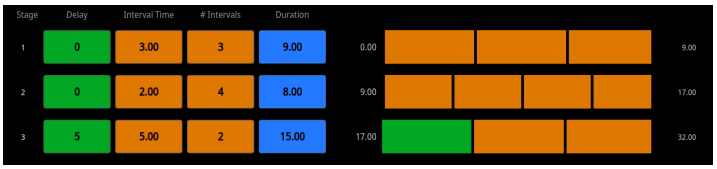
- Basic Instrument Operations

# Settings

These settings are available for creating kinetics methods.

| Tab | Setting | Description |
|---|---|---|
| Name and Settings | Method name | **Enter a name** for this method (this name appears in the Kinetics Setup box after the method has been saved). |
| | Description | Enter a detailed **description** for this method, if desired, such as the type of samples, added reagents, etc. |
| | Measurement range | **Select the spectral range** in which this method will acquire data. Available options:<br>• Ultra-violet only (190 nm - 350 nm)<br>• Visible only (350 nm - 850 nm)<br>• Ultra-violet and visible (190 nm - 850 nm)<br>• Custom range (specify starting and ending point in nanometers)<br>**Note**: For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent. |
| | Monitored wavelengths | **Enter up to 3 wavelengths** to be measured and reported at run time.<br>**Note**: All specified wavelengths must fall within the selected measurement range. |
| | Pathlength: | Enter cuvette pathlength (width) before taking blank or sample measurements with cuvettes (see cuvette manufacturer for cuvette specifications) |
| | Stir Speed: | If using automatic stirring, drop micro-stir bead into sample cuvette and set Stir Speed (levels 1 through 9 correspond with range from 10 RPM to 850 RPM with controlled ramping from zero) |
| | Heat cuvette to 37 °C: | Select this option if sample cuvettes require heating.<br>Cuvette heater increases from room temperature to 37 °C at rate of 5 °C/minute |

| Tab | Setting | Description |
|---|---|---|
| Stages and Intervals | Number of Stages | **Specify up to 5 stages** for kinetic measurements. Each stage can have unique Delay, Interval Time and # Intervals settings.<br><br>**Note**: Many kinetic measurements include only one stage. Additional stages are necessary only when a variation in stage interval or duration is needed. |
| | Time Unit | Select the **unit** for time-based measurements (seconds or minutes). |
| | Stage 1, 2, etc. | Specify the available settings for each stage:<br><br>• **Delay**. Specify a delay before a stage starts.<br><br>• **Interval Time**. Specify the length of time between measurements taken during this stage (minimum is 2 seconds). The first measurement occurs when the stage starts (or after the delay is completed if a Delay is specified).<br><br>**Note**: If two or more stages are specified with Delay set to zero, two measurements occur at the same time (the measurement at the beginning of the new stage directly overlaps the one at the end of the previous stage).<br><br>• **# Intervals**. Specify the number of absorbance measurements to take in this stage.<br><br>**Note**: Since the first measurement is taken when the stage starts, the number of measurements reported for each stage will be the **# Intervals** setting plus 1.<br><br>• **Duration**. Readout shows the total time required for this stage, including any delay and all specified intervals. |

| Tab | Setting | Description |
|---|---|---|
| | | The colored **rows** at the right (see image below) show the start and end times for each stage; the colored **boxes** at the right correspond with the specified delay and number of intervals for each stage.<br>  <br><br>If no delay is specified, absorbance measurements are taken at the start and end of each stage and after each specified interval. If a delay is specified, as in stage 3 above, the first measurement occurs at the start of the first interval. If the unit is seconds in the example above, a total of 11 measurements are taken at the following times over a period of 32 seconds: |
| | | • Stage 1: 0, 3, 6 and 9 seconds<br>• Stage 2: 9, 11, 13, 15 and 17 seconds<br>• Stage 3: 22, 27 and 32 seconds<br><br>**Note**: Kinetic experiments are limited to 1000 measurements. This means the total number of measurements from all intervals in all stages must be less than 1000. Consider available instrument or computer disc space for lengthy experiments. |

## Related Topics

- Instrument Settings

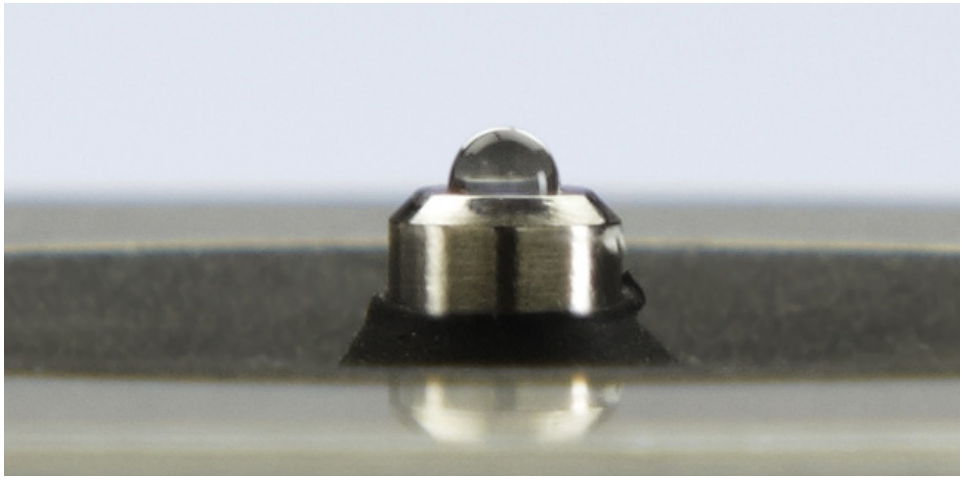
9

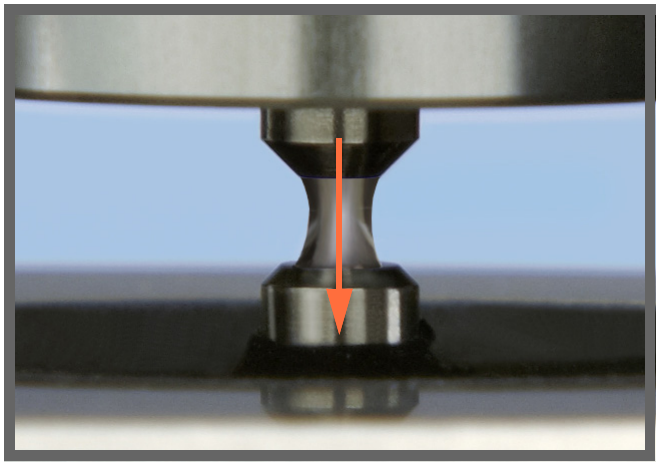
# Learning Center

**Contents**

# Micro-Volume Sampling—How it Works



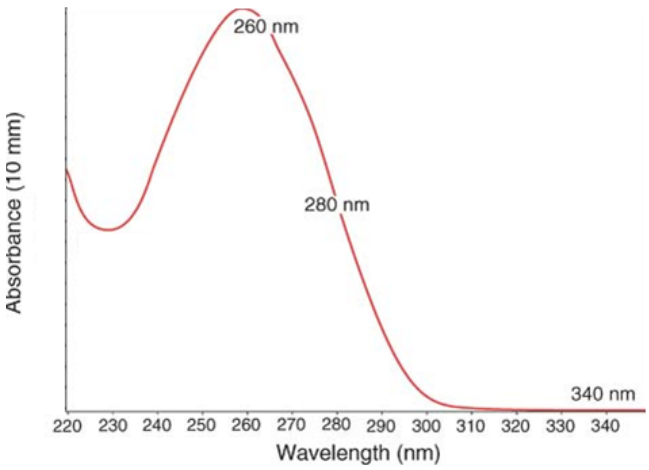




## Surface Tension

The NanoDrop Ultra Spectrophotometers and Fluorometers use surface tension to hold a small volume of sample between two pedestals. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

A fiber optic cable embedded in the upper pedestal leads to a xenon light source. A second cable embedded in the lower pedestal leads to a detector. When the instrument arm is down, the sample forms a liquid column, essentially bridging the gap between the two fiber optic cables.




$$\text{Absorbance} = -\log\left[\frac{\text{intensity}_{\text{sample}}}{\text{intensity}_{\text{blank}}}\right]$$

### Beer-Lambert equation

$$A = \varepsilon \cdot b \cdot c$$

where:

**A** = absorbance in absorbance units (A)

ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) in liter/mol-cm

**b** = pathlength in cm

**c** = analyte concentration in moles/liter or molarity (M)

## Absorbance Spectrum

The light passes through the liquid column to the detector, which generates a spectrum of absorbance versus wavelength. The spectrum shows the amount of light absorbed by the molecules of the sample at each measured wavelength.

**Note**: To prevent evaporation, which affects measurement accuracy, close the arm quickly after you finish loading a sample or blank.

The example at the left shows a typical absorbance spectrum taken of a nucleic acid sample. The spectrum is measured from 190 nm to 850 nm. The displayed range may vary for each application.

## Sample Absorbance

When the instrument is blanked, a reference spectrum is taken of the blanking solution and stored in memory. For each sample measurement, the sample intensities along with the blank intensities are used to calculate the total absorbance of the sample according to the equation at the left.

## Sample Concentration

The Beer-Lambert equation (Beer's law) shown at the left is used to correlate sample absorbance with concentration.

The pathlength is the distance between the two pedestals, which varies in real time during each measurement. This auto-ranging pathlength technique produces accurate concentration results over a wide dynamic range.

### Baseline Correction

For some applications, the instrument can be set up to apply a baseline correction to each measurement to minimize any offset caused by light scattering particulates in the sample spectra. The correction subtracts the absorbance value at a reference wavelength that is close to zero from the absorbance value at each wavelength across the spectrum, essentially “anchoring” the spectrum to zero absorbance units at the reference wavelength.

### Sample Relative Fluorescence

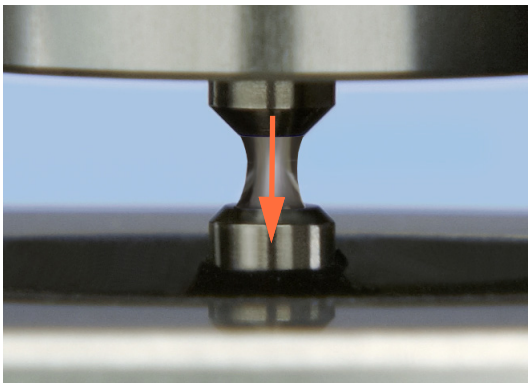
The Red or Blue LED is used as the light source, the light passes through an excitation filter, through the liquid column, through an emission filter to the detector, which records relative fluorescence of the sample.

### Fluorescence Sample Concentration

A curve-fitting algorithm which requires a minimum of two standards is used in the calculation of concentration data. The NanoDrop Ultra dsDNA BR, dsDNA HS, and RNA HS Fluorescence Assays all use a modified Hill plot.

# Measure a Micro-Volume Sample

The NanoDrop Ultra spectrophotometers and fluorometers use surface tension to hold a small volume of sample between two pedestals. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions. Tap here for details.



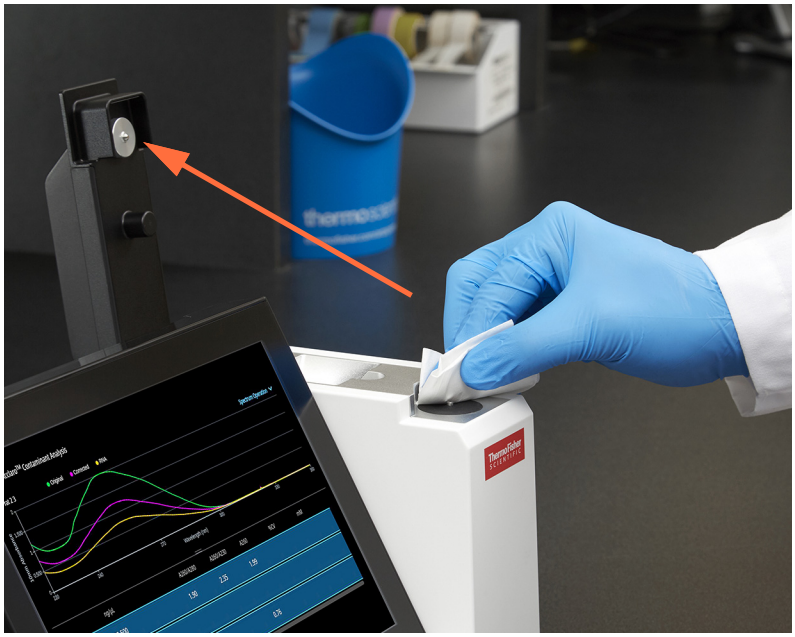
### Supplies needed

- NanoDrop Ultra Spectrophotometer (Ultra, Ultra$^{C}$, Ultra FL, Ultra$^{C}$ FL)
- lint-free laboratory wipes
- calibrated precision pipettor (0–2 µL)
- sample material resuspended in appropriate buffer solution (see Preparing Samples)
- pure buffer solution for blanking instrument (see Choosing and Measuring a Blank.

## Best practices for micro-volume measurements

### Cleaning pedestals for daily operation

- Before first measurement, clean both pedestals with a new laboratory wipe.
- Run a blanking cycle to verify pedestals are clean.
- After each measurement, clean both pedestals with new wipe to prevent carryover.
- After each set of measurements, clean pedestals with DI H2O (see Clean pedestals between users)
- Recondition pedestals periodically to maintain their hydrophobic property.



### Pipetting Samples

- Use recommended sample volumes to ensure proper liquid column formation.
- Use calibrated precision pipettor (0–2 µL volume range) with well-fitting, low-retention precision tips to apply sample material to instrument for measurement.

  If using low accuracy (0-10 µL) pipettor, use 2 µL sample volumes.
- Filter tips are not recommended as filter particulates can impact absorbance measurements at 230 nm
- Use new tip for each blank and sample aliquot.
- Use new aliquot of sample for each measurement.
- When the measurement is complete, open the sampling arm and wipe the samples from both the upper and lower pedestals using a soft laboratory wipe.
- If solvents are used, make sure they are compatible with the pedestals. (see “Compatible Solvents” in Hazardous Materials).

## Recommended sample volumes

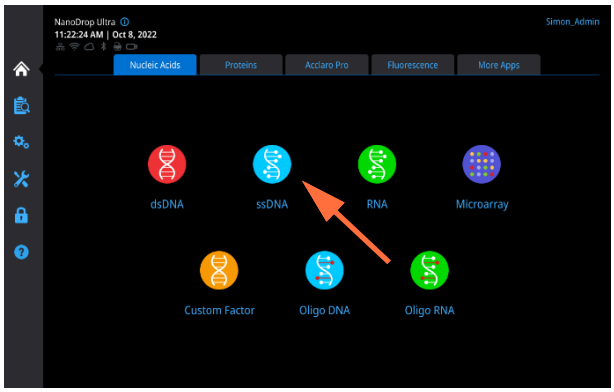
| Application | Sample Volume |
|---|---|
| Nucleic acid (aqueous solution) | 1 µL[a] |
| Purified protein | 2 µL |
| Other protein applications such as Bradford or BCA | 2 µL |
| Microbial cell suspensions | 2 µL |
| Fluorescence | 2 µL |

[a] Use 2 µL for samples that contain materials that may reduce surface tension such as a surfactant.

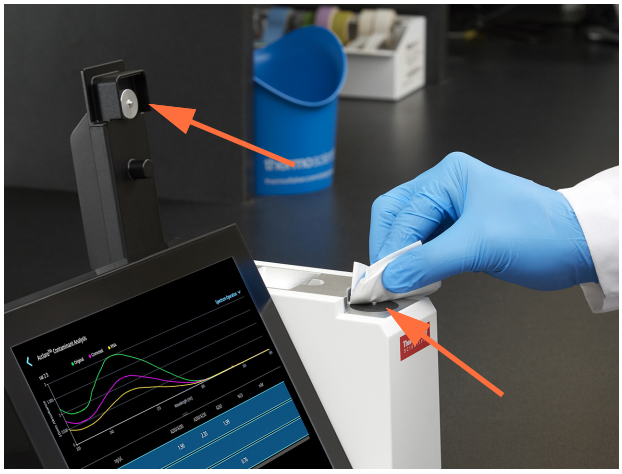
## To measure a micro-volume sample

**NOTICE**

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.



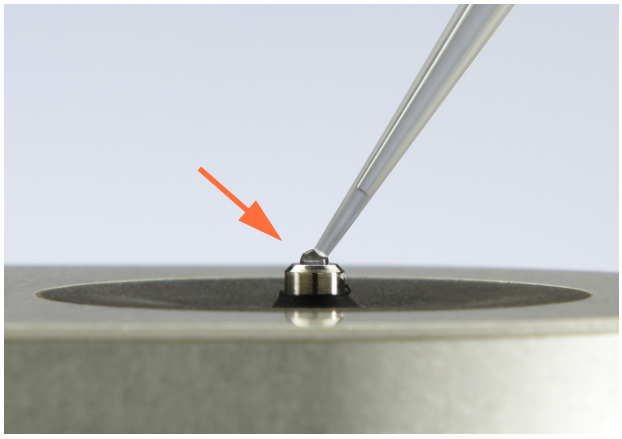

1. From the instrument home screen, select an application from one of the application categories, such as **dsDNA**.



2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.

3. Measure a blank:
   - Pipette 1–2 µL blanking solution onto the lower pedestal and quickly lower the arm
   - Select **Blank** and wait for the measurement to complete

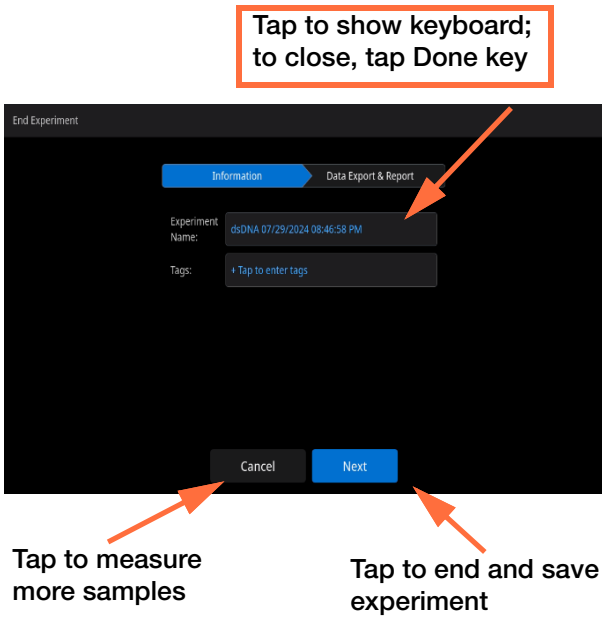
     **Tip**: If Auto-Blank is On, blank measurement starts automatically after you lower the arm.
   - Lift the arm and clean both pedestals with a new laboratory wipe




**Tap to end experiment**

4. Measure the first sample:
   - Pipette 1-2 µL sample solution onto the pedestal and quickly lower the arm (see Recommended Sample Volumes for more information)
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is Off, lower arm and select **Measure**
   - When the sample measurement is completed, the spectra and reported values are displayed.
5. To measure another sample:
   - Lift the arm
   - Clean both pedestals with new wipe
   - Load the next sample and quickly lower the arm
   - Start the sample measurement
   - Wait for the measurement to complete

   The new spectrum replaces the previous one on the spectral display and the new reported values appear under the previous ones in the table. (When measuring many samples, press and hold the chart area while moving up or down to show information for samples that may be offscreen.)




6. When you are finished measuring samples:
   - Select End Experiment (see previous image)
   - Enter an experiment name (tap **Experiment Name** box to display keyboard), or leave the default experiment name
   - Select **Next**.
   - Lift the arm and clean both pedestals with a new wipe

     If finished with the instrument for the day, clean the pedestals with DI H2O (see Clean pedestals between users)

Acquired data are automatically saved in an experiment with the entered name. In the default configuration, experiments are stored in a database on the local instrument according to acquisition date, experiment name, application used and any assigned labels (see Manage identifiers on the instrument).

# Measure a Sample Using a Cuvette

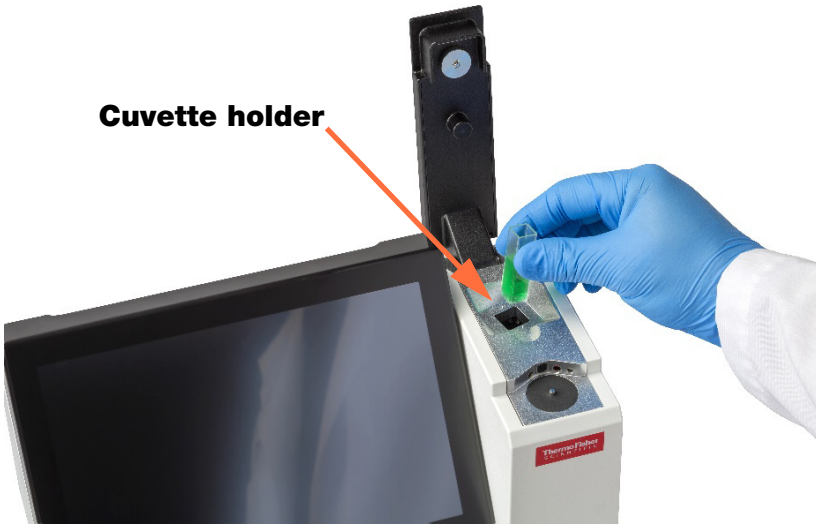
The NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL spectrophotometer models include a cuvette holder for measuring dilute samples, colorimetric assays, cell cultures and kinetic studies. The cuvette system offers an extended lower detection limit and an optional 37 °C heater and micro-stirrer.


### Supplies needed

- NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL
- lint-free laboratory wipes
- two compatible cuvettes
- sample material resuspended in appropriate buffer solution (see Preparing Samples)
- pure buffer solution for blanking instrument (see Choosing and Measuring a Blank.)

## Best practices for cuvette measurements

- The instrument arm can be up or down for cuvette measurements.
- Use 10 mm, 5 mm, 2 mm or 1 mm cuvettes.
- Clean and dry cuvette after each measurement.
- Use cuvettes that are free of scratches and avoid fingerprints which may affect results.
- Use quartz cuvettes or UV-grade plastic cuvettes to measure samples with analysis wavelengths in the UV range (<340 nm).
- Micro, semi-micro, and ultra-micro cuvettes should be masked.
- Fill cuvettes with enough blanking or sample solution to cover instrument optical path (2 mm sample beam is 8.5 mm above cuvette bottom).
- Lift instrument arm and make sure cuvette holder is free of debris.
- When inserting quartz or masked plastic cuvettes, align cuvette light path with instrument light path.

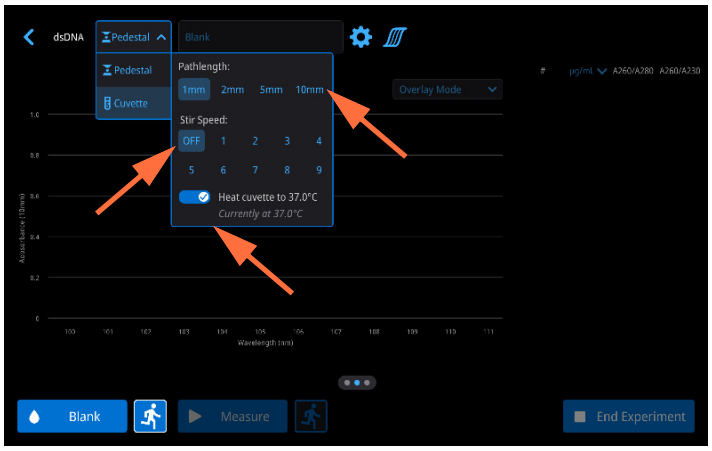



### To measure a sample using a cuvette

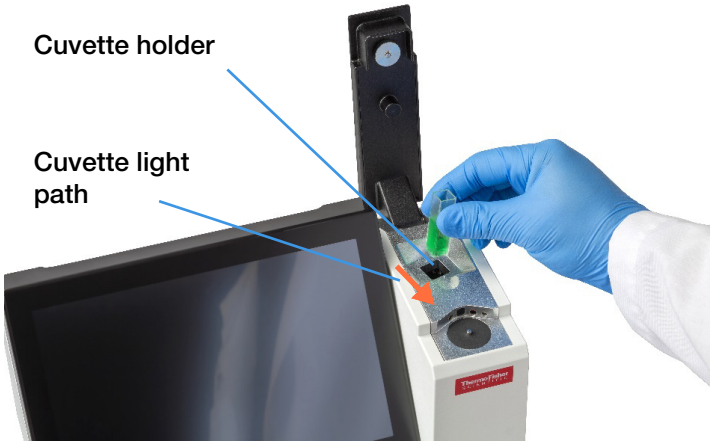
**NOTICE**

- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.

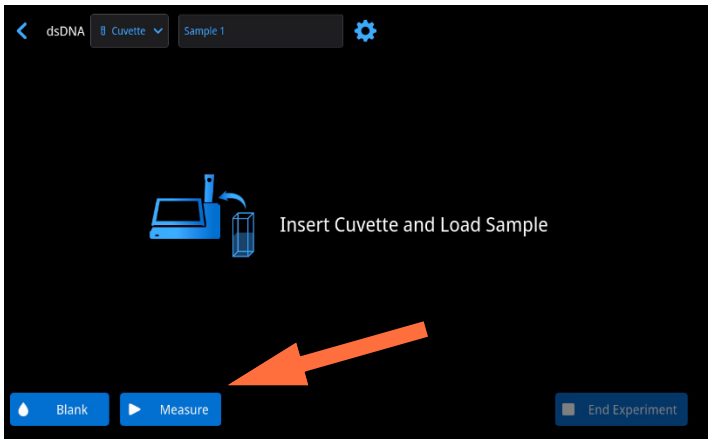

1. Open one of the applications and select **Save**.
2. The dropdown will default with **Pedestal** selected, open the dropdown and select **Cuvette** to switch to the Cuvette mode. The dropdown must then be closed for the change to take effect.




3. Specify the cuvette options:
   - Set Pathlength to pathlength (width) of cuvette (see cuvette manufacturer for specifications)
   - Set Stir Speed and heater if desired
   - Select anywhere outside of the box, to close the window




4. Measure a blank:
   - Fill clean, dry cuvette with enough blanking solution to cover instrument optical path
   - Lift instrument arm and insert blanking cuvette into cuvette holder, making sure to align light path of cuvette with light path of instrument
   - Select **Blank** and wait for the measurement to complete




5. Measure a sample:
   - Fill clean cuvette to same height with sample solution
   - Replace blanking cuvette with sample cuvette, making sure to align light paths
   - Select **Measure**
   - Wait for measurement to complete
   - Remove cuvette
   - Clean cuvette according to manufacturer specifications

## Cuvette Settings

Select cuvette sampling mode. When selected, these additional options are available:

**Pathlength**: Enter cuvette pathlength (width) before taking blank or sample measurements with cuvettes (see cuvette manufacturer for cuvette specifications)

**Stir Speed**: If using automatic stirring, drop micro-stir bead into sample cuvette and set Stir Speed (levels 1 through 9 correspond with range from 10 RPM to 850 RPM with controlled ramping from zero)

**Heat cuvette to 37 °C**: Select this option if sample cuvettes require heating. Cuvette heater increases from room temperature to 37 °C at rate of 5 °C/minute.

# Prepare Samples and Blanks

## Preparing Samples

- Isolate and purify samples before measuring them with the instrument. Commercial sample isolation kits are available for these purposes, or use an in-house protocol. After purification, analyte of interest is typically dissolved in aqueous buffer solution before it is measured.

  **Tip**: Any molecule that absorbs light at analysis wavelength will contribute to total absorbance value used to calculate sample concentration.

- Ensure final analyte concentration is within instrument's absorbance detection limits.

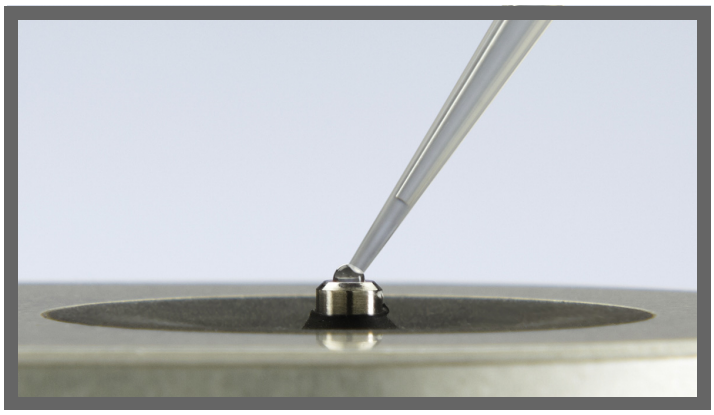
- For micro-volume measurements, gently (but thoroughly) vortex each sample before taking a measurement.

Avoid introducing bubbles when mixing and pipetting.

**Note** Samples dissolved in extremely volatile solvent such as hexane may work best with cuvette sampling option (NanoDrop Ultra and NanoDrop Ultra$^{C}$ FL instruments only).

## Choosing and Measuring a Blank

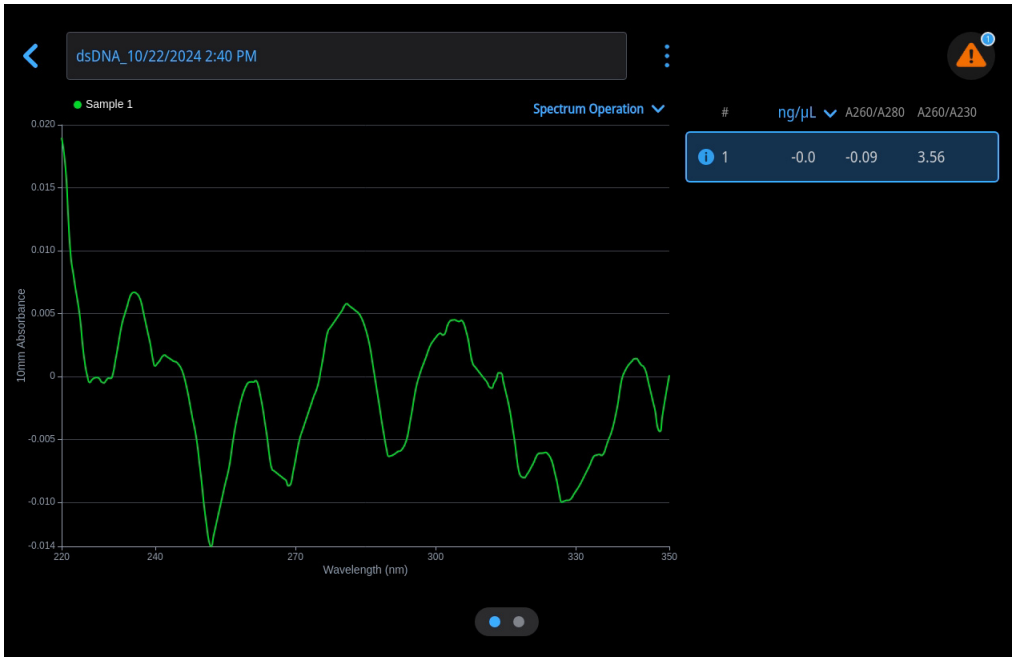
The buffer used to resuspend a sample analyte can contribute absorbance. Blanking minimizes any absorbance contribution due to the buffer components from the sample measurement. The resulting sample spectrum represents the absorbance of only the analyte of interest.



**For best results:**

- For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Measure new blank before each set of samples. It is not necessary to blank the instrument before each sample measurement unless the samples are dissolved in different buffer solutions.
- Measure a new blank every 30 minutes.
- Run a blanking cycle to assess the suitability of your blanking solution before using it to perform sample measurements.

The resulting spectrum should vary no more than 0.02 A or 0.4 A (10 mm equivalent) depending on the application across the spectrum, especially at the analysis wavelength as in the example below.

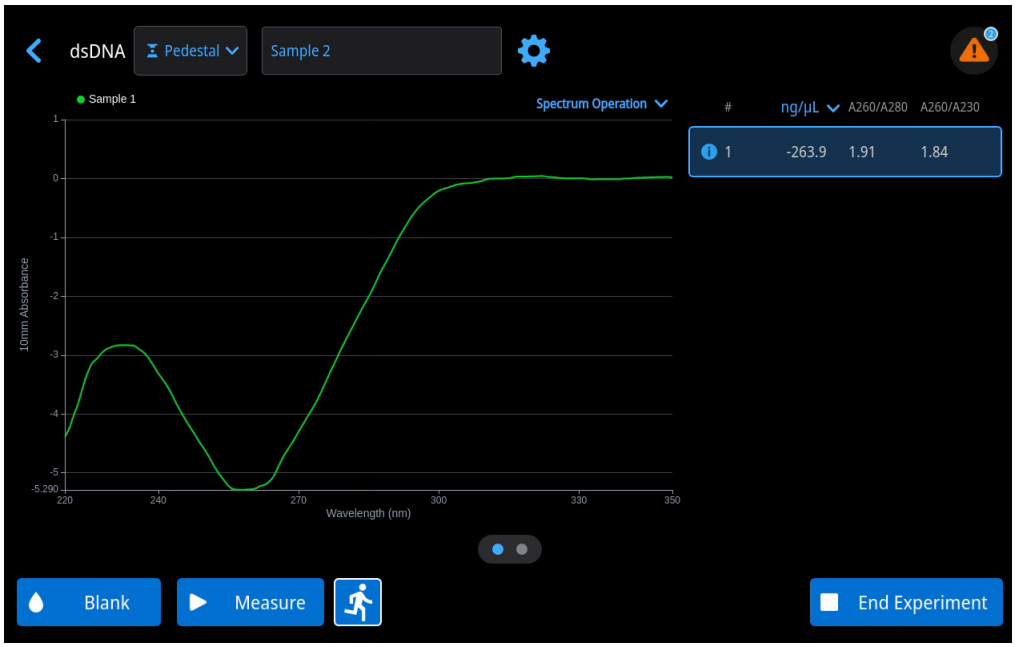



**Good blanking buffer (measured abs < 0.02)**

If the resulting spectrum is greater than 0.02 A or 0.4 A (10 mm equivalent) depending on the application around the analysis wavelength, that buffer solution may interfere with the sample analyses, especially for low concentration samples. See below for details.

## Problems associated with blanking

- Residual sample was left on pedestal or in cuvette before blank measurement was performed. (Resulting sample spectra may exhibit negative absorbance values, indicating blank had more absorbance than sample in that region of spectrum.)
- Blank measurement exhibits higher absorbance than unknown sample at analysis wavelength. (If buffer used as blank differs in composition from that used to resuspend sample, measurement results will be incorrect.)
- Sample was inadvertently used to blank instrument. (Resulting sample spectra may exhibit negative absorbance values or, in some cases, resemble a mirror image of a typical pure nucleic acid or protein spectrum as in example below.)




**Nucleic acid sample solution used to blank instrument results in "mirror image" spectrum**

### Solutions for blanking problems

- Thoroughly clean and/or recondition both pedestals and then:
  - rerun blanking cycle, or
  - measure new blank using new aliquot of appropriate buffer solution, then measure new aliquot of unknown sample

For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.

# Run a Blanking Cycle

Run a blanking cycle to verify the following:

- instrument is operating normally (with flat baseline)
- pedestals are clean (i.e., no dried-down sample material on pedestals)
- absorbance contribution of buffer solution you plan to use for sample analyses

### Supplies needed

- lint-free laboratory wipes
- 0–2 µL calibrated precision pipettor (low retention tips)
- buffer solution for evaluation

### To run a blanking cycle

**NOTICE**

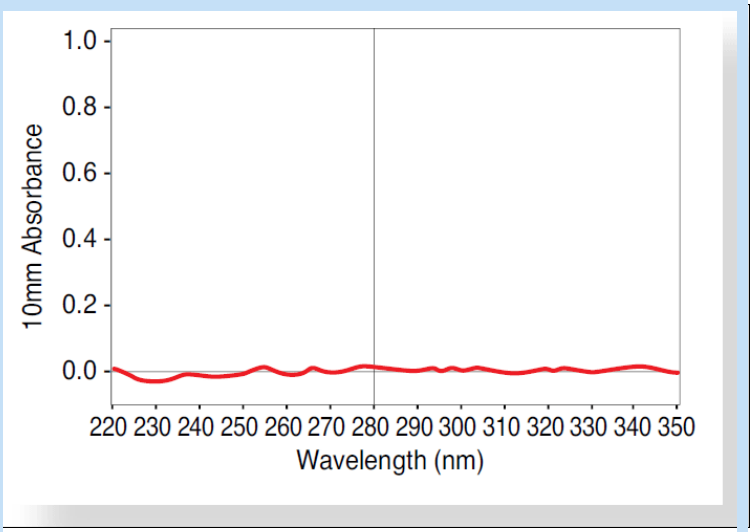
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

1. From the home screen, select an application and save the settings.
2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.
3. Measure a water blank:
   - Pipette exactly 1 µL deionized water (DI $H_2O$) onto the lower pedestal and lower the arm.
   - Select **Blank** and wait for the measurement to complete.
   - Lift the arm and clean both pedestals with new laboratory wipe.
4. Measure the buffer solution:
   - Pipette 1-2 µL buffer solution onto the pedestal and lower the arm.
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and select **Measure**
   - Wait for measurement to complete.

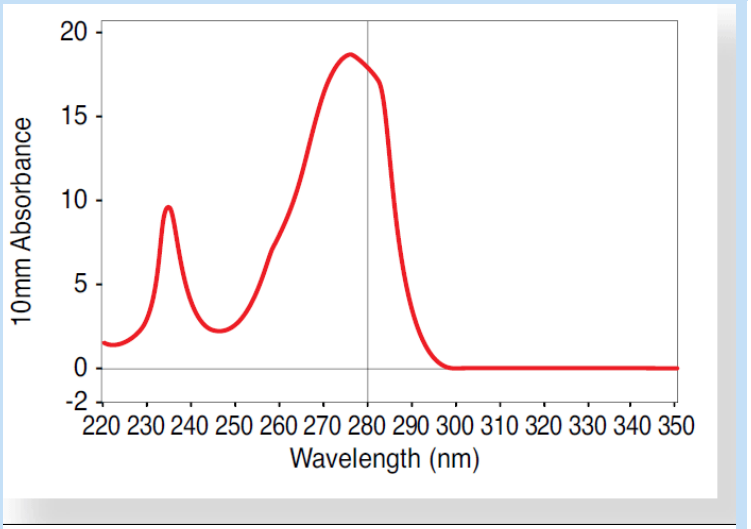
   The resulting spectrum should vary no more than 0.02 A (or 0.04 depending on the application used) from the baseline at the analysis wavelength.

   If your spectrum does not meet these criteria, repeat steps 2–4.

   If spectrum is still outside specifications, see Solutions for Blanking Problems.
5. When you are finished with the blanking cycle, select **End Experiment**.
6. Lift the arm and clean both pedestals with a new wipe.




Example spectrum of buffer suitable for Protein A280 protein quantification



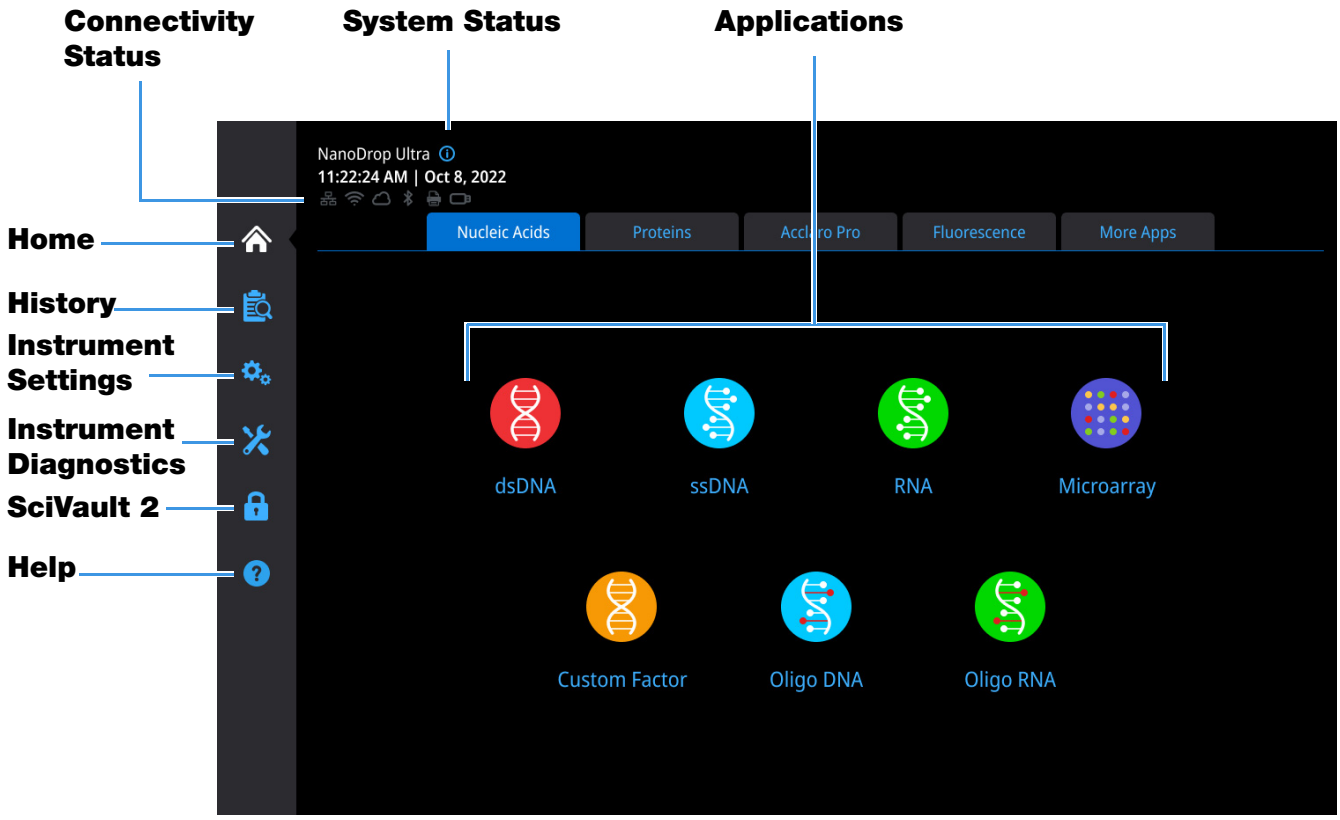

Example spectrum of buffer unsuitable for Protein A280 protein quantification

# Basic Instrument Operations



## NanoDrop Ultra Home Screen

These operations are available from the NanoDrop Ultra home screen.

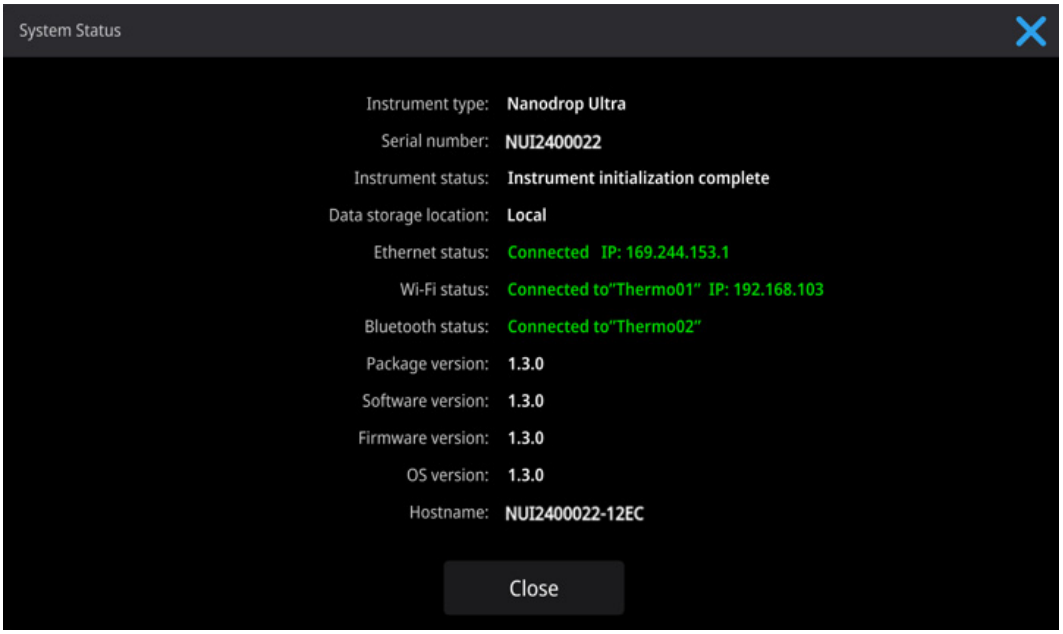



### Applications

The NanoDrop Ultra software offers several configurable applications, which gives users full control of the measurement.

## System Status

Select  on the instrument home screen to open the system status box.




The available information is described below.

| | |
|---|---|
| Instrument type | Instrument model (NanoDrop Ultra) |
| Serial number | Instrument serial number |
| Instrument status | Current status of the instrument |
| Data storage location | Indicates location of database set where instrument is currently storing data. |
| Ethernet status | Status of Ethernet connections for the instrument (“Connected to…”, or “Not connected”) |
| Wi-Fi status | Status of WiFi connections for the instrument (“Connected to...”, “Enabled and not connected”, or “Disabled”) |
| Bluetooth status | Status of Bluetooth connections for the instrument (“Connected to...”, “Enabled-[list of any paired devices]”, or “Disabled”) |
| Package version | Version of entire package that is installed on the system. Helps manage dependencies and ensures compatibility between firmware, OS, and software versions |
| Software version | Version of instrument operating software installed |
| Firmware version | Version of instrument firmware installed |

| | |
|---|---|
| OS version | Version of customized Linux operating system software installed |
| Hostname | Unique identifier of instrument including serial number and last 4 digits of MAC address |

## Connectivity Status

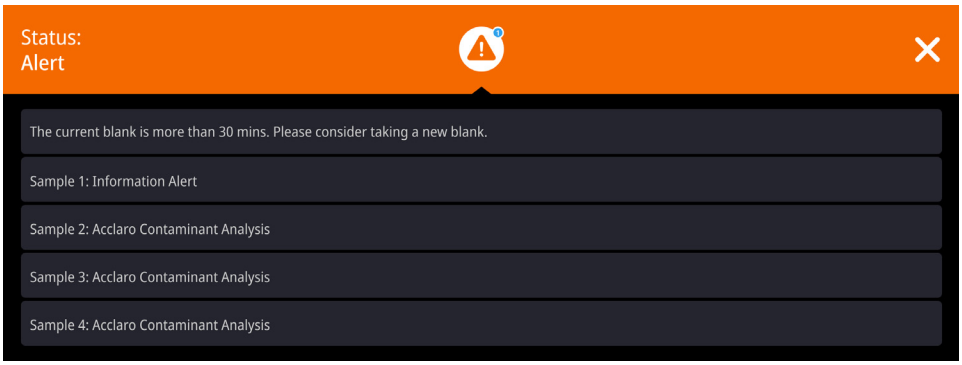
| | | |
|---|---|---|
| Ethernet Connectivity |  | Shows that the instrument is successfully connected to an Ethernet network. |
| Wi-Fi Connectivity |  | Shows that the instrument is successfully connected to a Wi-Fi network. |
| Bluetooth Connectivity |  | Shows that the instrument is successfully connected to a Bluetooth device. |
| Printer Connectivity |  | Shows that the instrument is successfully connected to a printer. |
| USB Connectivity |  | Shows that the instrument is successfully connected to a USB device. |

## System Alarms and Alerts


The NanoDrop Ultra software will alert users of any potential issues, available software updates, or if any Acclaro Sample Intelligence information is available during an experiment. Depending on the severity of the notification, either the alert icon  or the alarm icon  will be displayed at the top of the screen. Both icons

contain a blue circle with a number indicating the amount of alert or alarm messages that are available. A detailed explanation will appear of all status alerts or alarms by selecting their respective icon. Selecting some individual alerts or alarms may prompt additional information.

The software will also display a banner that provides additional information about the alert or alarm at the time it was triggered. Selecting the banner will provide you additional information.




The alert and alarm banners can be minimized by selecting to the right of the message.

The alert for software updates will only occur if the instrument is connected to a network with internet access.

### Navigation Bar

The following options makeup the Navigation Bar. The Navigation Bar can be found on the left side of the screen at various times in the software including on the home screen.

### Home Screen

Select to return to the home screen.

### History

Select to view any data acquired earlier today, last week, last month, last six months, last year or in a specific date range. See “View History” on page 315 for more information about the History feature on the instrument.

### Instrument Settings

Select to access instrument settings for software updates, cuvette sampling, networking and more. See “Instrument Settings” on page 338 for detailed information about all available instrument settings.

### Instrument Diagnostics

Select  to verify instrument operation. Instrument diagnostics should be run periodically according to the recommended maintenance schedule. See “Instrument Diagnostics” on page 363 for information about how to run the available instrument diagnostics.

### SciVault 2

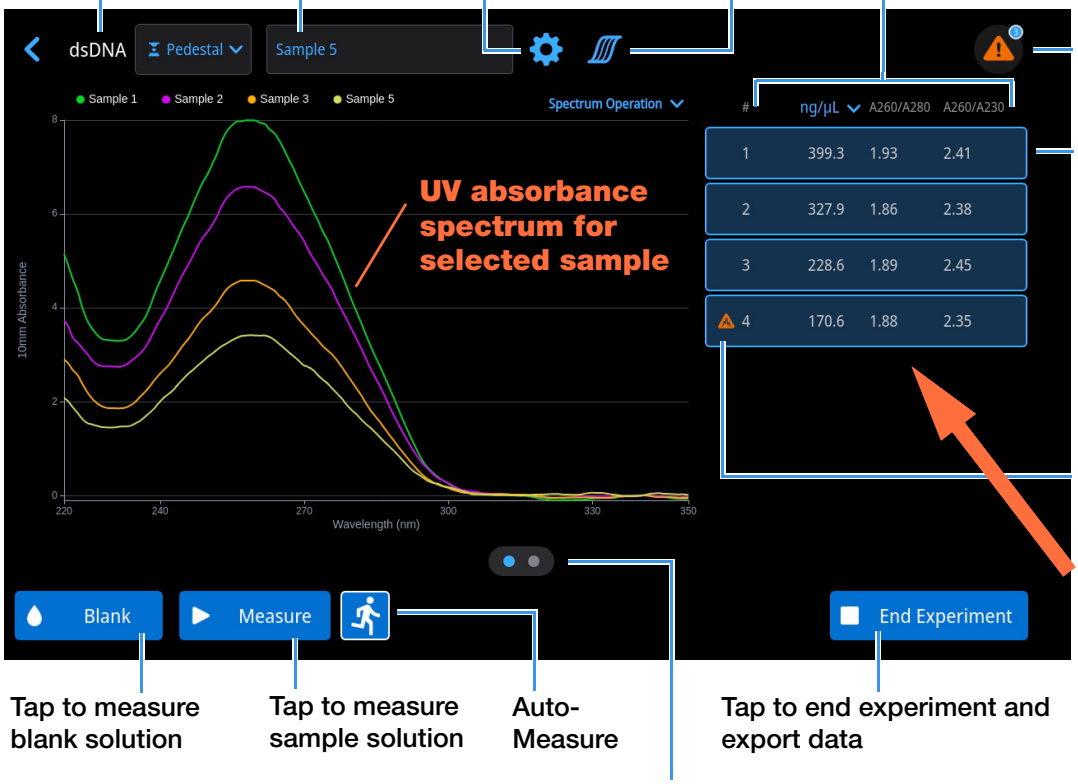
Select  to open the optional add-on Thermo Scientific™ SciVault™ 2 software. This companion software allows users to operate their NanoDrop Ultra instrument in a manner that complies with US FDA 21 CFR Part 11. When purchased, the SciVault 2 software and activation key is shipped to you on a USB stick and integrates directly into the NanoDrop Ultra software user interface. For more information, visit thermofisher.com/nanodrop.

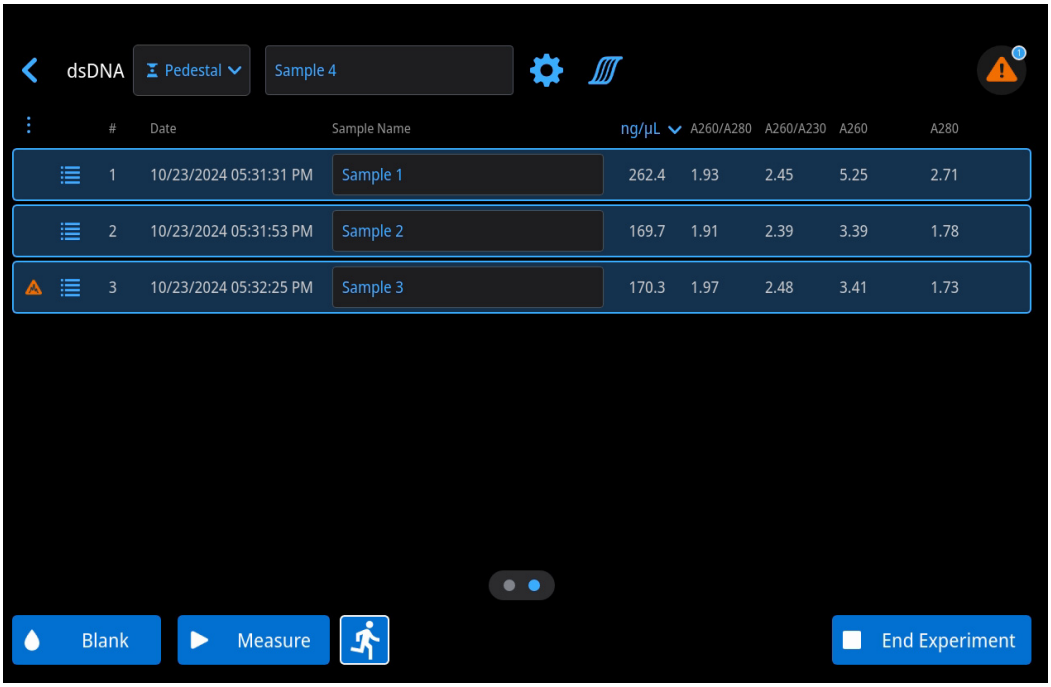
### Help

Select  to launch the interactive help guide.

# NanoDrop Ultra Measurement Screens

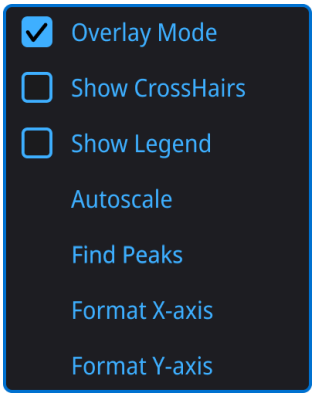
These operations are available from any measurement screen within an Application.






## Measurement Screen Display Options

When performing measurements, right-click the graph on the measurement screen or select the Spectra settings icon Spectrum Operation to bring up the following display options:




**Display multiple samples in overlay.**

**Hover spectrum to see plot data**

**Display graph legend**

**Scale axes to fit spectra measurement**

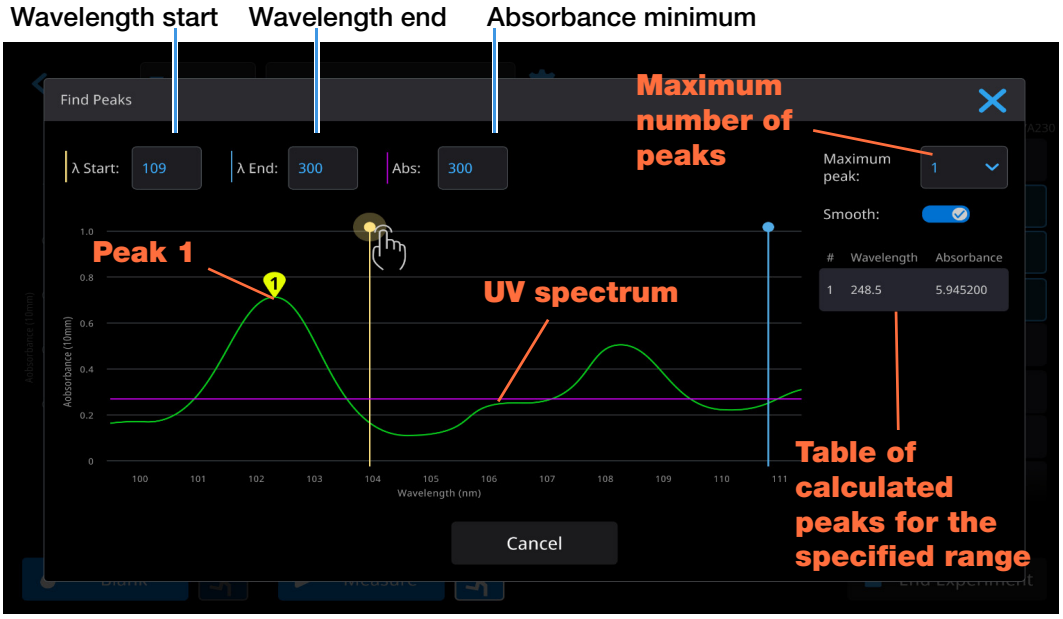
**Calculate peaks for specified range**

**Select to manually enter x-axis range**

**Select to manually enter y-axis range**

## Find Peaks

Select **Find Peaks** to view calculated peaks for specified range. You can enter the range by dragging the color-coded limit lines, or enter values into the fields at the top of the spectrum. Found peaks for the defined range are listed in the table below the spectrum.




## Setup

Select  in any measurement screen to see the available setup options for that application.

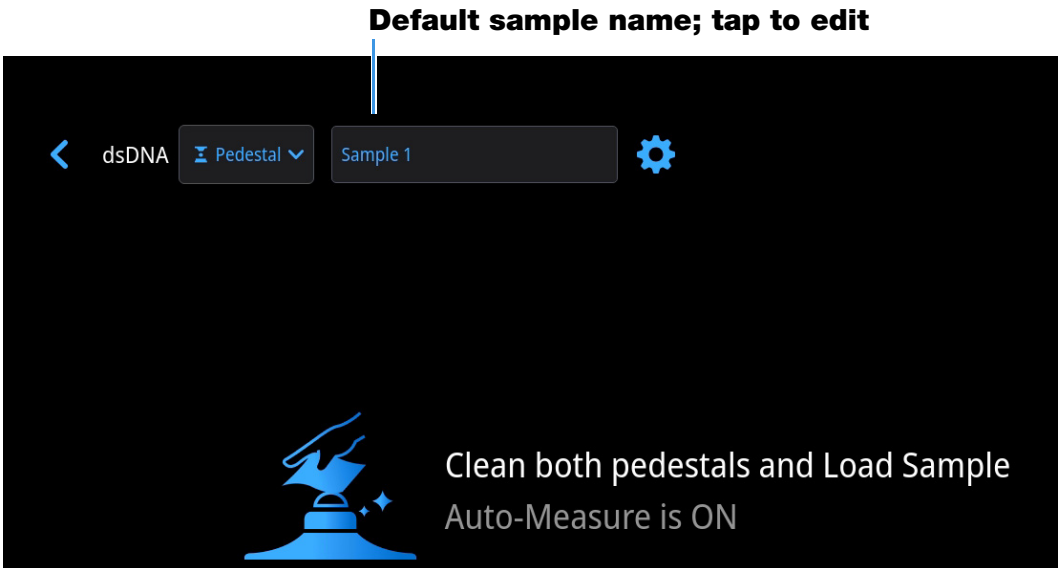
The types of setup options that appear in the setup screen depend on the selected application. For details, see the settings section of that application in this guide:

Applications > [application group] > Settings

## Sample Name

Select the **Sample Name** field in any measurement screen to edit the sample name.

When Auto-Naming is enabled in the application setup screen, each sample is automatically assigned a sample name using the default base name followed by a unique number starting with "1." The first time this appears is after the first blank measurement and before the first sample measurement in each experiment as shown below.




In this example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name.

**Note** If you edit the sample base name during an experiment when Auto-Naming is selected, the assigned sample ID numbers restart.

### Edit default sample base name

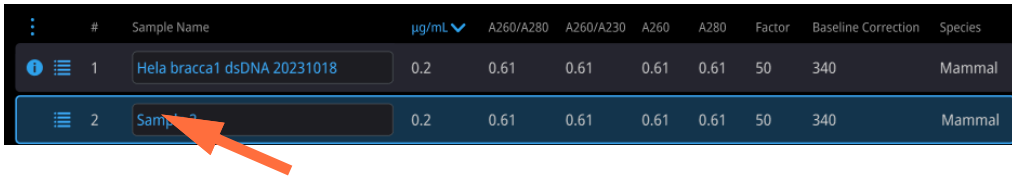
After you measure a blank and before the first sample is measured:

1. Select **Sample Name** field to display keyboard
2. Enter new base name
3. Select **Done** key

### Edit sample name

1. From home screen, select  to open History

2. Select experiment
3. **Swipe left** to show data table
4. Select the **Sample Name** field to display keyboard




5. Enter new sample name
6. Select **Done** key to close keyboard

## Measurement Results

The types of results that appear in the measurement screens depend on the selected application. For details, see the reported results section of that application in this guide:

Applications > [application group] > Measure [application name] > Reported Results

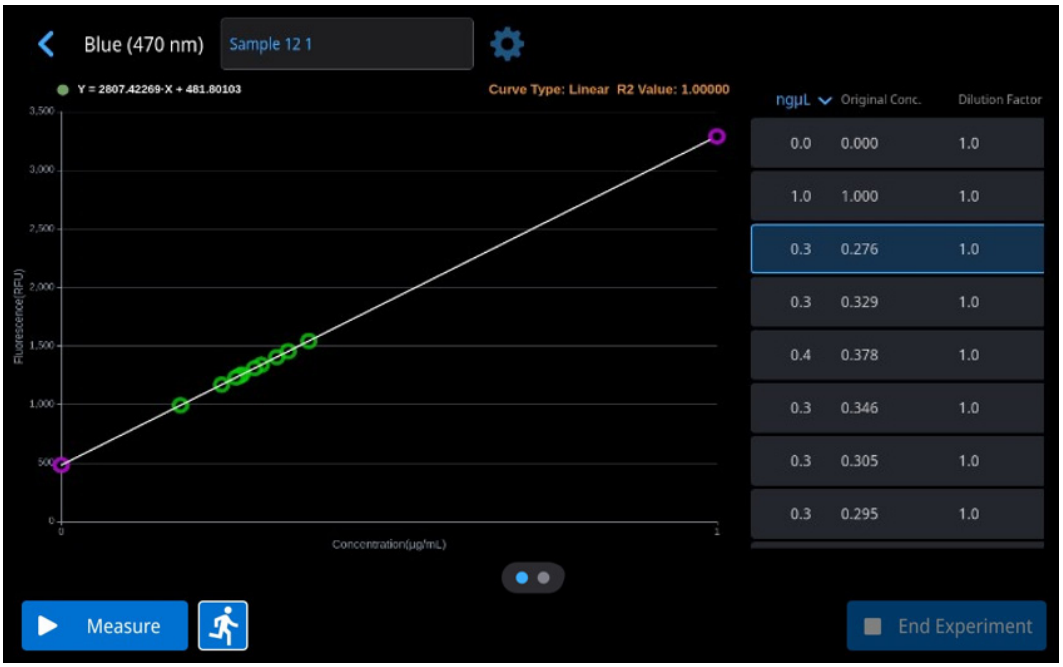
## Absorbance Spectrum

For each measured sample taken in one of the applications (Fluorescence applications not included), the UV or UV-Visible absorbance spectrum and a summary of the results is shown. The vertical axis shows absorbance in absorbance units (A). The horizontal axis shows wavelength in nm. Here is an example for a UV-Vis method.

All absorbance-based applications display the sample pathlength along the spectrum's vertical axis. Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent. Here is an example.


## Fluorescence Standard Curve

For each measured sample taken in one of the Fluorescence applications, a standard curve and a summary of the results is shown. The vertical axis shows fluorescence in relative fluorescence units (RFU). The horizontal axis shows concentration in ng/µL. Here is an example from the dsDNA Fluorescence application.




## Acclaro Measurement Alerts



The Acclaro Sample Intelligence technology built into the NanoDrop Ultra instruments provides important features to help you assess sample integrity. Tap or click a Sample Intelligence icon in the software to view its associated information.

contaminant analysis is available to help qualify a sample before use in downstream applications

on-demand technical support is available for measurements that are atypical or very low concentrations

## Blank Button

Select **Blank** to measure a blank for the selected experiment.

A blank must be measured before each group of similar samples. The blank solution is typically the pure buffer that was used to resuspend the sample. For more information, see Choosing and Measuring a Blank.

## Measure Button

Select **Measure** to measure a sample for the selected experiment.

Samples must be properly isolated and prepared before they can be measured with the instrument and the concentration must be within the instrument's absorbance detection limits. For more information, see Preparing Samples. and Measure a Micro-Volume Sample or Measure a Cuvette Sample and Detection Limits.

**Note** The **Measure** button is enabled after a valid blank measurement is completed.

## Auto-Measure and Auto-Blank Options

Speed up sample analysis with the NanoDrop Ultra Auto-Measure and Auto-Blank features, which cause the instrument to start the measurement immediately after you lower the instrument arm. These options eliminate the need for repetitive Measure or Blank operations for large batches of samples.

**Note** Auto-Measure and Auto-Blank are available for micro-volume measurements only.

### Auto-Measure

To select or deselect Auto-Measure, from any sample measurement screen, select the **On** or **Off** button at the right of the Measure button.


### Auto-Blank

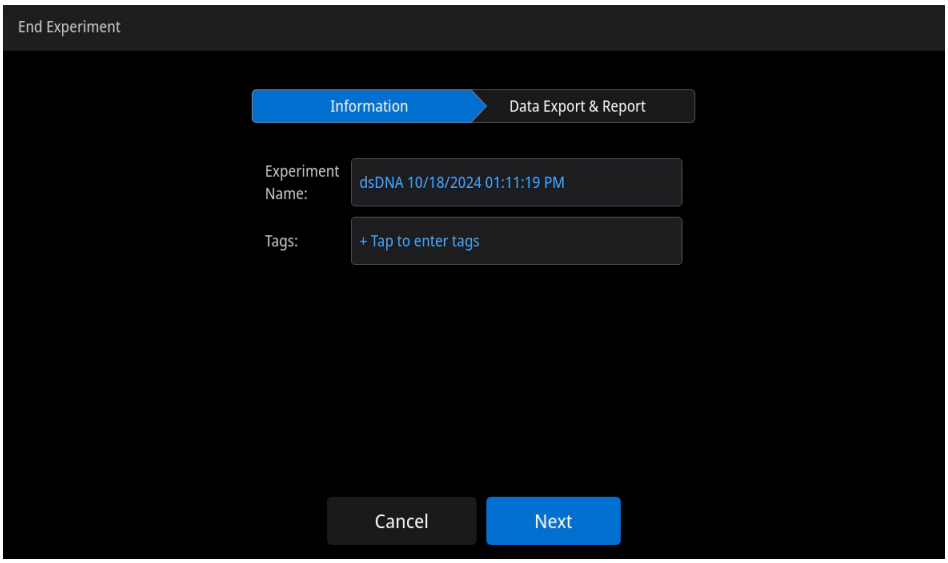
To select or deselect Auto-Blank, from any blank measurement screen, select the **On** or **Off** button at the right of the Blank button.

## End Experiment Button

Select **End Experiment** when you are ready to name and save your experiment, add a label to help you locate the experiment later, print, or export the data. Depending on the administrative settings, you may be prompted to sign the experiment upon ending the experiment.

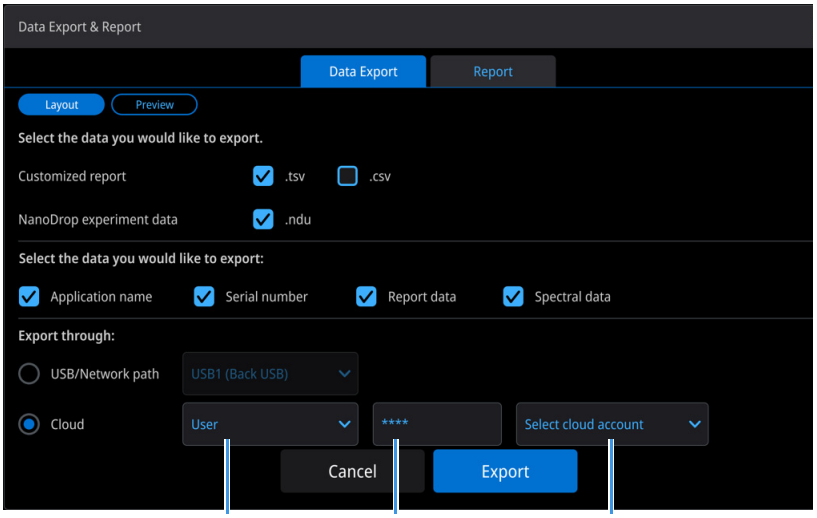

**Note** The **End Experiment** button is enabled after the first sample measurement is completed.

After you select End Experiment, the information tab within the End Experiment box is displayed:



**Available options:**

| | |
|---|---|
| Experiment Name | Enter a name for this group of measurements. The measurement results are saved in the selected database location using the entered experiment name. |
| Tags | Enter a descriptive label to help you find this experiment later or to associate it with another experiment (see Manage identifiers on the instrument for details).<br><br>When using the local control software, Tap the **Tags** box to display a keyboard to enter the label text.<br><br>Press the enter key (PC control) or **Done** (local control) to add the label. |
| Export Data | Select **Next** to move to the Data Export & Report tab within the End Experiment window.<br><br>Select **Continue** to open the Data Export & Report window |




**Thermo Fisher User Account** **Thermo Fisher PIN** **Available Cloud accounts**

### Available options (Data Export):

| | |
|---|---|
| Export File Type | The first section allows you to select a file format for exporting the measurements in this experiment.<br><br>Available export file formats:<br><br>• Customized report<br>– comma-separated values spreadsheet (.csv) file<br>– tab-separated values spreadsheet (.tsv) file (spectral data only)<br>• NanoDrop Ultra experiment data (.ndu) file<br>– tab-separated values spreadsheet "TQ Analyst" (TQ) file used for troubleshooting purposes<br><br>The filename is the entered experiment name (see above). The file is stored in a folder named "NanodropUltra" followed by the instrument serial number. (Use **System Status** to view your instrument serial number.) |
| Customized Report Options | The second section is dedicated to what information will appear within one of the customized report options (.csv or .tsv).<br><br>Available customizable options:<br><br>• Application name - when enabled will include the name of the application used.<br>• Serial number - when enabled will include the serial number of the instrument that was used during the experiment.<br>• Report data - when enabled will include information from all columns currently displayed in the report table"<br>• Spectral data - when enabled will include absorbance data for displayed spectrum in 0.5 nm increments. |

Export Location

The last section is dedicated to where the export file will be saved.

**USB/Network Path–**

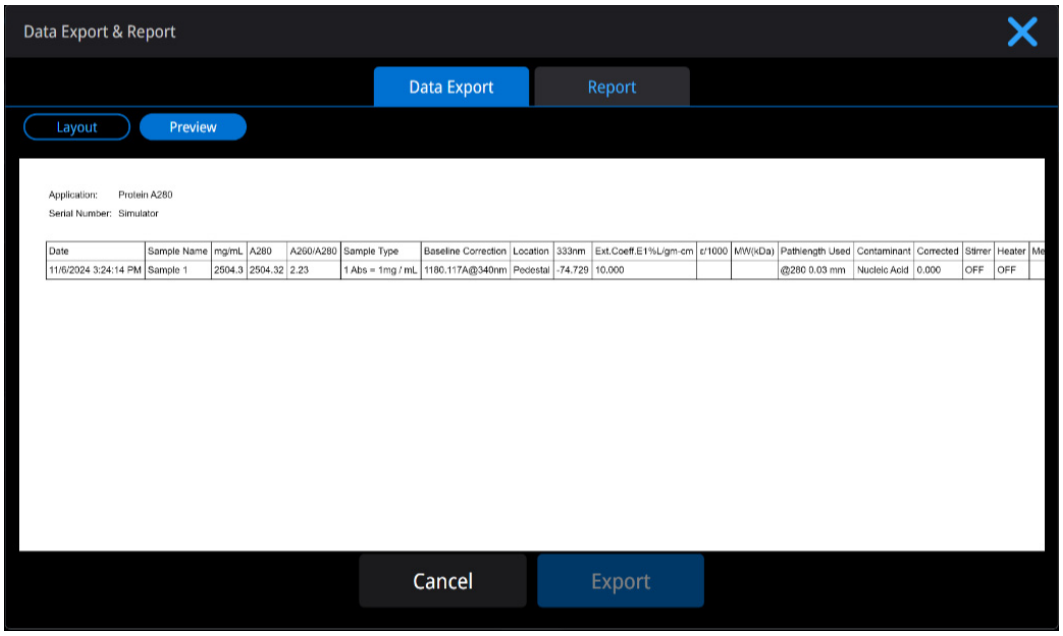
Select the USB/Network path radio button to export to a USB device connected to any USB port on the local instrument (front, back-left or back-right), or to a network location. When using the NanoDrop Ultra PC control software, use the Browse option to select a File Path to export to.

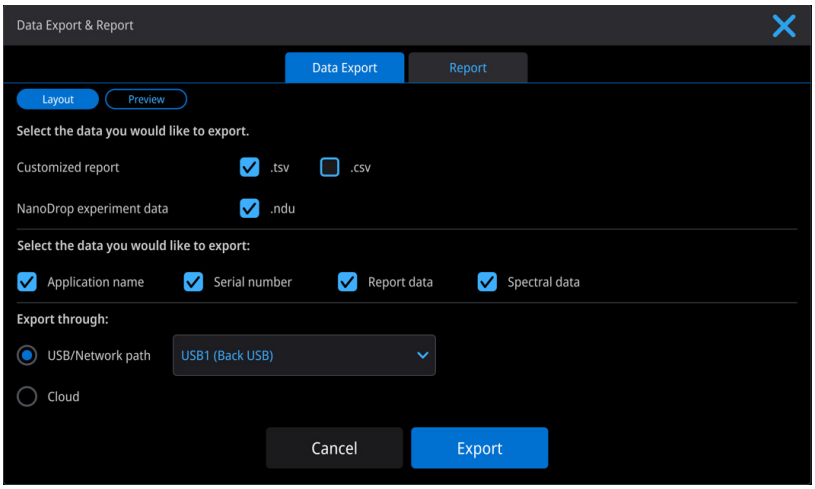
**Cloud–**

Select the Cloud radio button to export to one of the available Cloud options: Thermo Fisher Cloud Connect™, Google Drive, or Microsoft OneDrive (see Cloud for more details). The Thermo Fisher Scientific Cloud Connect™ username and PIN will need to be selected/entered before finally selecting the available Cloud location to export to.

Export Preview

Select the **Preview** tab to see what the exported file will look like including any customization that was selected. This feature will not work for the .ndu export file type.



| | |
|---|---|
| Cancel (Return to Experiment) | Close the **End Experiment** box and display the results for the most recent measurement. From there you can add measurements to the current experiment and save it later. |
| Export Button | Export measurement results for current experiment.  |

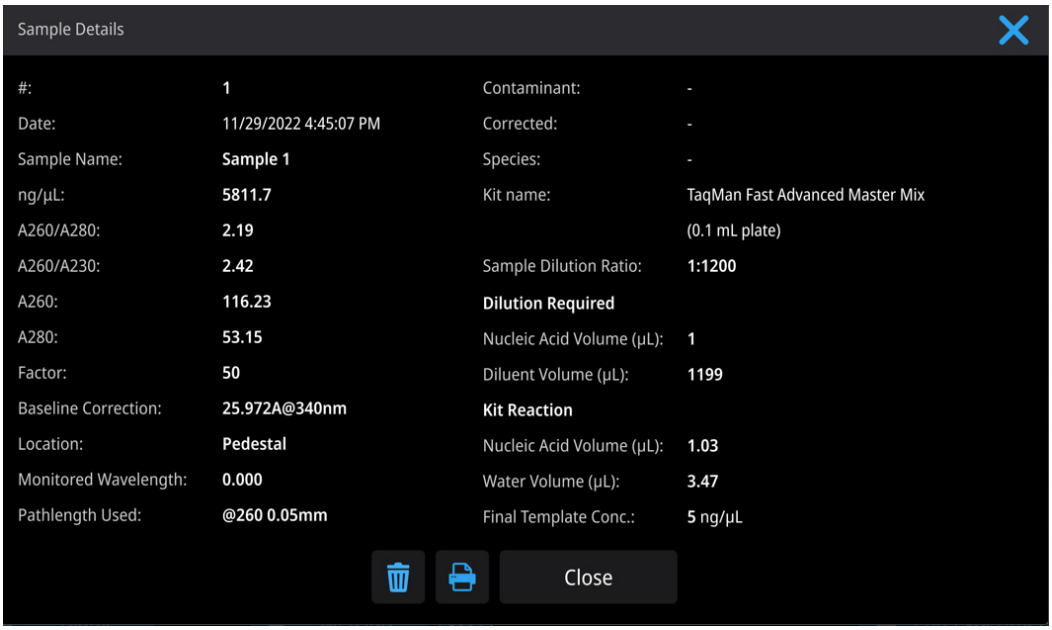
## Available options (Report)

| | |
|---|---|
| Customized Report Options | Section dedicated to what information will appear within the printable report.<br><br>Available customizable options:<br><br>• Sample Spectra Display - option to overlay all sample spectra or display up to 4 sample spectra per page.<br><br>• Header/Footer Display - option to input a title and sub-title on the report to appear on all pages as well as enter any notes to appear at the end of the report.<br><br>• Report data - option to include information from specific columns currently displayed in the report table |
| Print to PDF Location | Section dedicated to where the export file will be saved.<br><br>Select the USB/Network path radio button to export to a USB device connected to any USB port on the local instrument (front, back-left or back-right), or to a network location. When using the NanoDrop Ultra PC control software, use the Browse option to select a File Path to export to.<br><br>Select the Cloud radio button to export to one of the available Cloud options: Thermo Fisher Cloud Connect™, Google Drive, or Microsoft OneDrive (see Cloud for more details). The Thermo Fisher Scientific Cloud Connect™ username and PIN will need to be selected/entered before finally selecting the available Cloud location to export to. |
| Print Preview | Select the **Preview** tab to see what the printable report file will look like including any customization that was selected.<br><br>  |

| | |
|---|---|
| Cancel (Return to Experiment) | Close the **End Experiment** box and display the results for the most recent measurement. From there you can add measurements to the current experiment and save it later. |
| Print button | Select printer to print from and print measurement results for current experiment. |
| Save to .pdf button | Export the .pdf report for measurement results for current experiment. |

## Sample Details

Press and hold a **sample row** in any measurement screen or data table to show the sample details, which include all available measurement results and associated details for the selected sample. Here is an example:

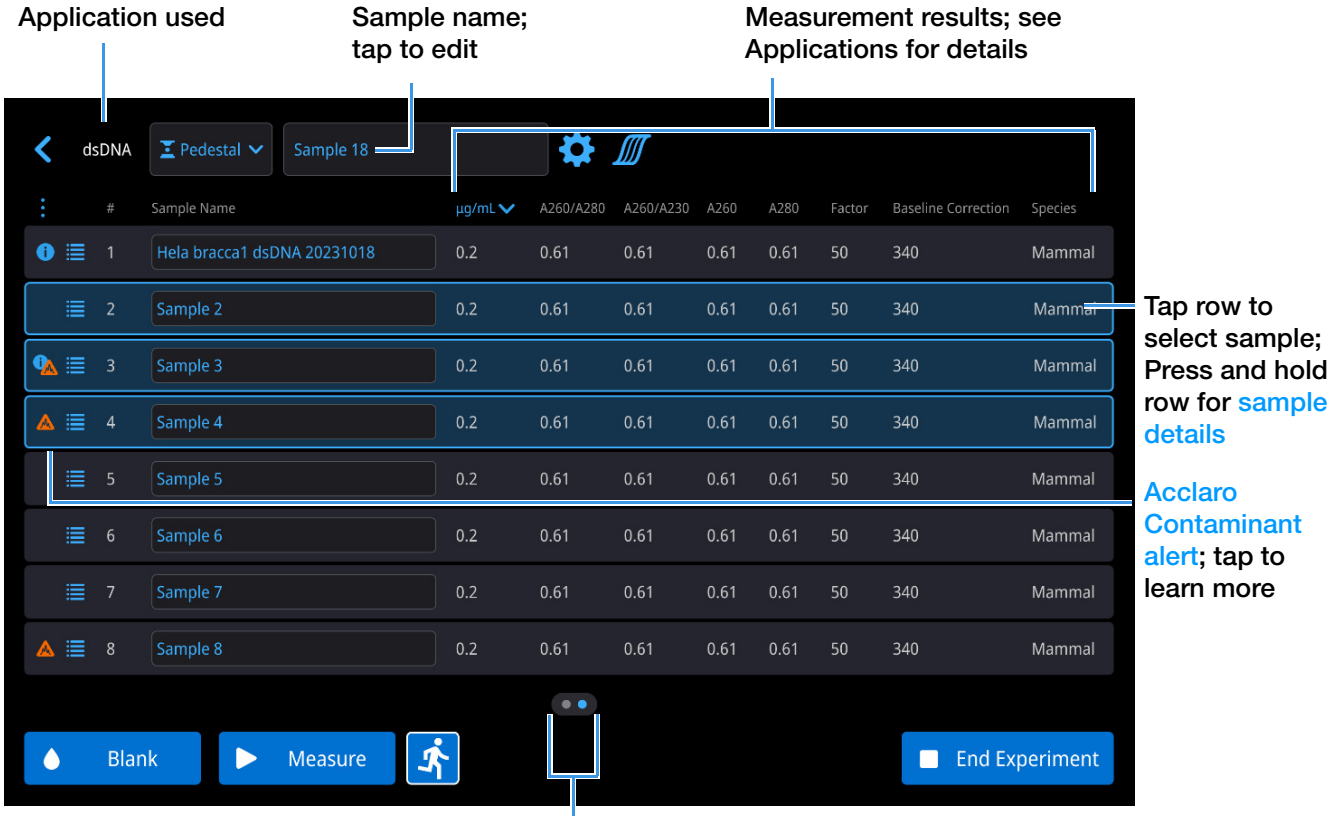



Information about the measured values displayed in Sample Details is provided in this Help system, under the application used to acquire the data.

**Note** You can also edit the sample name from the Sample Details box.

## Data Table

Swipe left in any measurement screen to see the data table for the current experiment. The data table contains the measurement results for all samples in the experiment. The image below highlights the available features.




## View History

Whether you collect one sample or many in a row, after you choose End Experiment, the acquired data are automatically saved in an experiment with an experiment name. In the default configuration, experiments are stored in the NanoDrop Ultra database on the local instrument according to acquisition date, experiment name, application used and any assigned labels.

Use the History feature to open the database in order to view acquired spectra and associated data from any experiment at any time.

### Open instrument database of measurement results

To open NanoDrop Ultra database on instrument, tap (History) on instrument home screen.

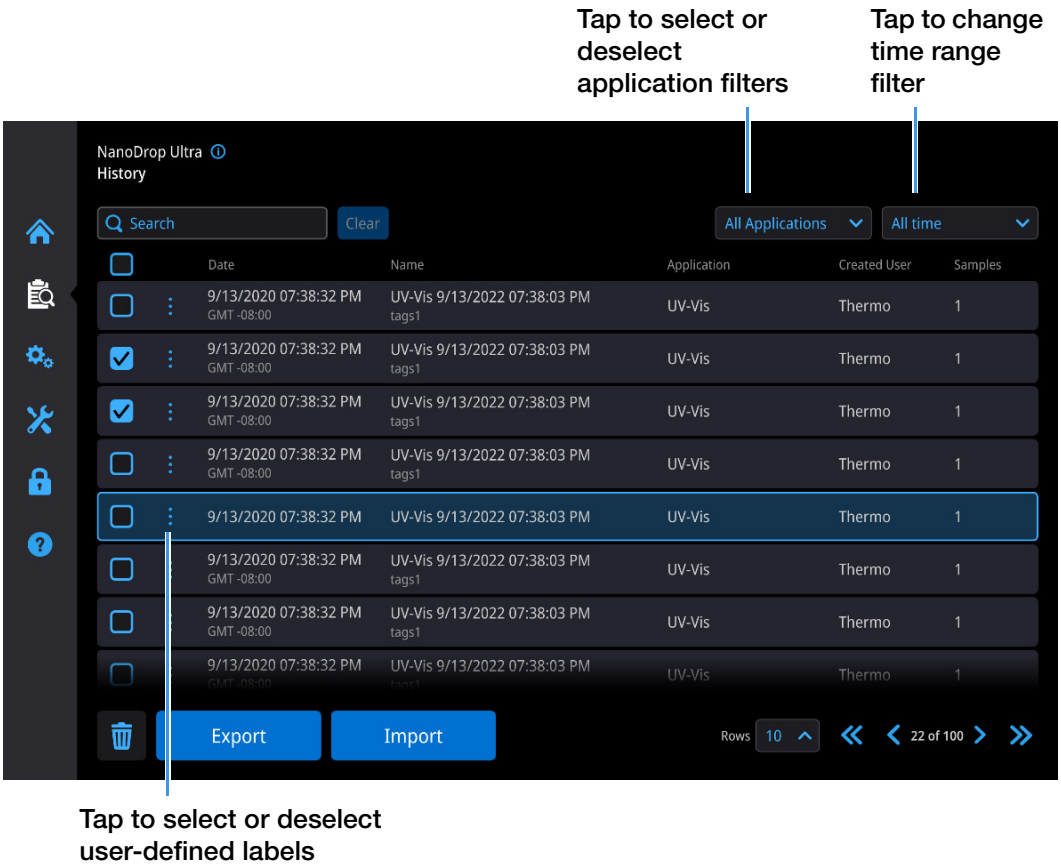
## History Menu

**Available options:**

| | |
|---|---|
| Delete | Delete the experiment |
| Import | Import data from a USB flash drive or folder on your computer or network hard drive (NanoDrop Ultra PC control software only) |
| Export | Export the experiment |

## Search Experiment Database

Use the **Search** feature in History to search the selected database for an experiment or to change the time range or other search filters. The database is filtered using the current settings in the Search box. Filters include time range, application type and any user-defined labels (see Manage Identifiers for information about adding and deleting labels). Here is an example:

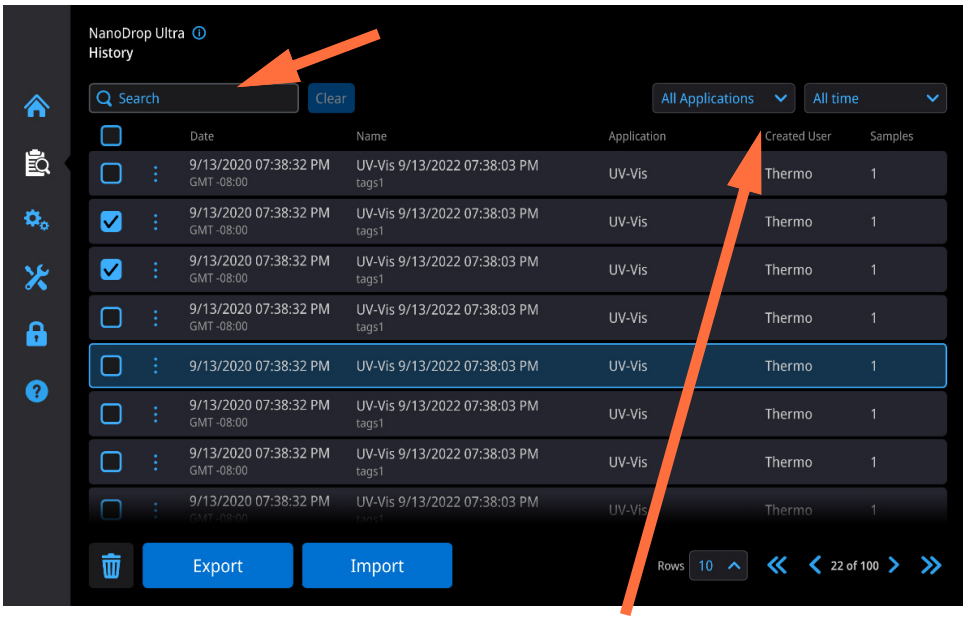


## Export Selected Experiments

To the left of each experiment an empty checkbox will appear. Select one or more checkboxes in History to select experiments to be exported.

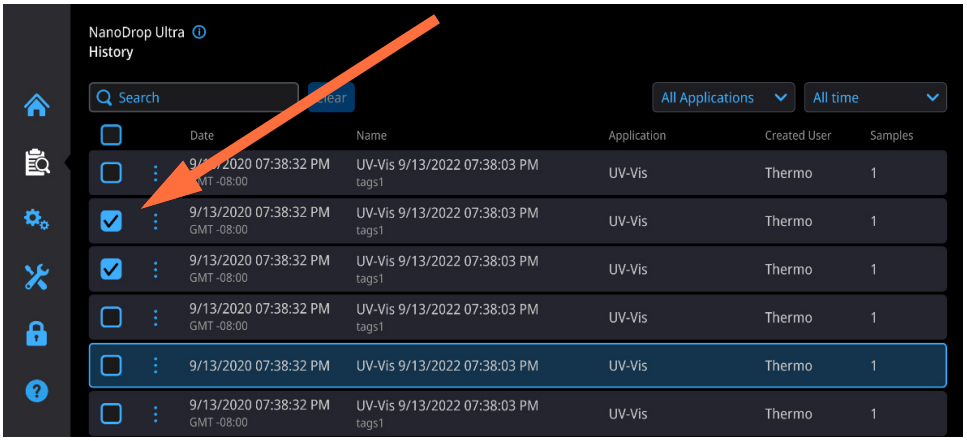
### Export selected experiments

– Within History, filter using the **Search** feature to find the desired experiment

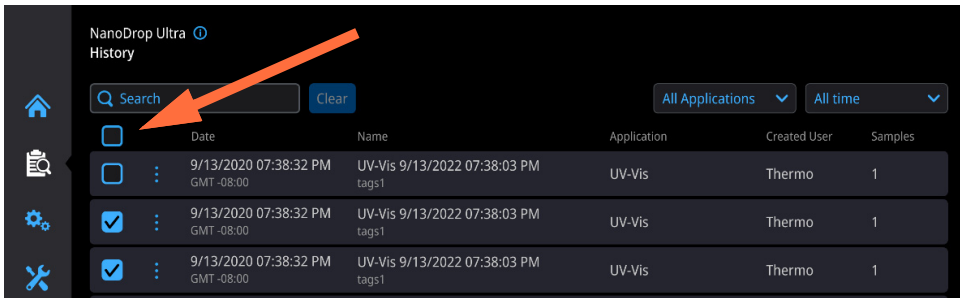



Change a filter to display updated list of experiments

– Click or tap the checkbox to select the row or rows containing the desired experiment(s) to be exported (click or tap again to deselect an experiment)

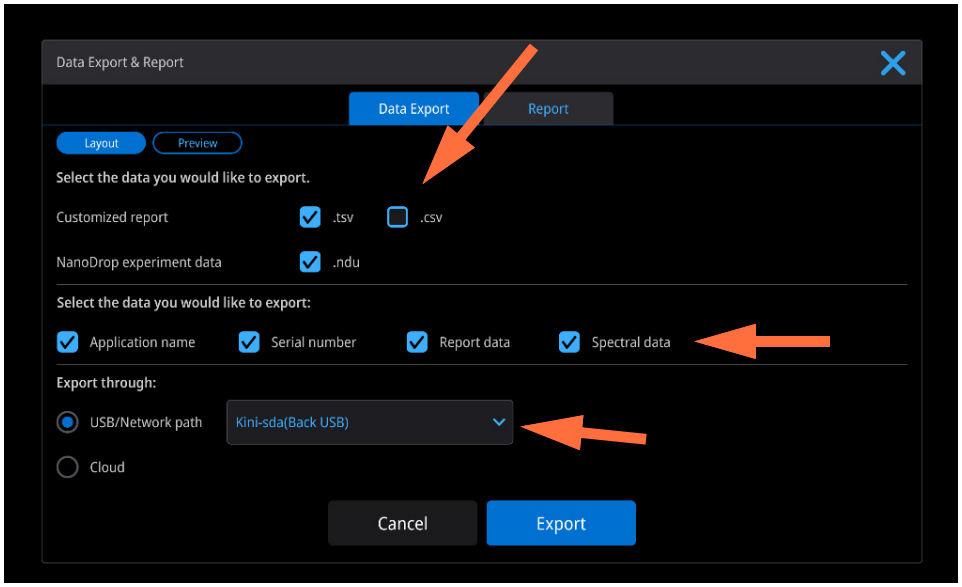


– To select or deselect all experiments in database, click or tap on the checkbox directly below the Search window




– Click or tap **Export**

– Select one or more formats to export to (see "Export Selected Experiments" on page 324)




– Select the applicable details to appear in the exported file

– Designate the export location and select **Export**.

– After "Export Completed" message, select **OK**

## Delete Selected Experiments

Select experiments to be deleted.

### Delete selected experiments

– Within History, filter using the **Search** feature to find the desired experiment

– Click or tap the checkbox to select the row or rows containing the desired experiment(s) to be deleted (click or tap again to deselect an experiment)

– Select  and **Yes**

**Note** Deleted data cannot be recovered.

## Open Experiment and View Associated Data

Use  (History) to locate and open any experiment to see the measurement data it contains.

### Open an experiment

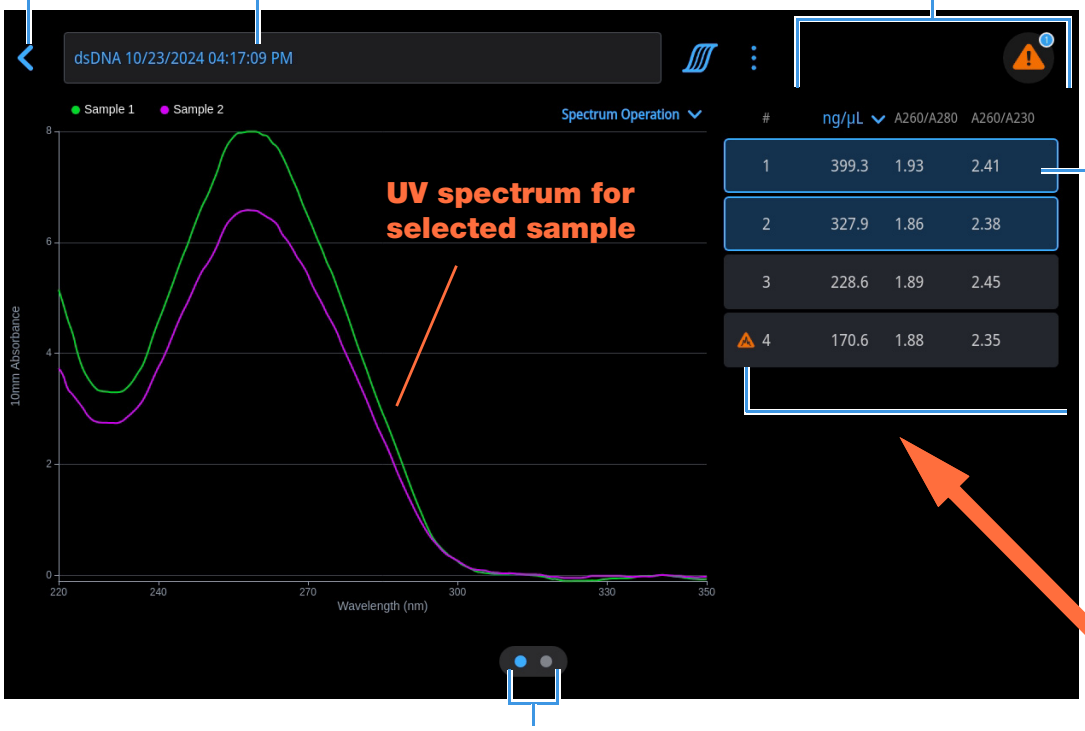
- Within History, if you don't see the experiment you want to open, you can use the Search feature to find the desired experiment
- Double click or tap the experiment name to open the experiment

The History provides measurement data as spectral data, standard curve data, and data tables, similar to what you see after you complete a measurement.

**Note** The data shown are dependent upon the application used to measure the samples (nucleic acids in these examples). For more information, see the application details.

### Spectral data—

After you open an absorbance-based experiment, the software shows the UV or UV-visible absorbance spectrum and a summary of the associated data for the first sample measurement, much like it appears during a measurement. The image below describes the available features.

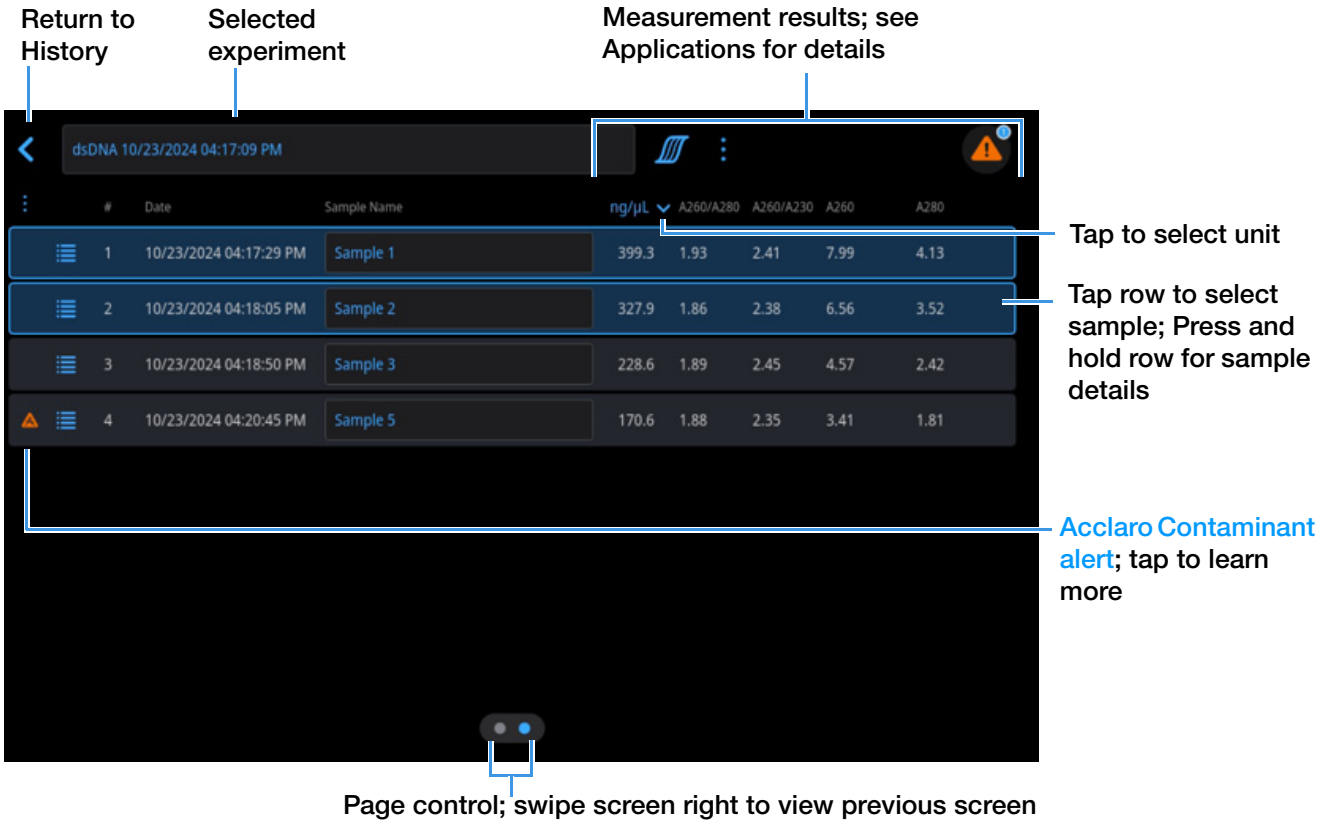



### Standard curve data—

After you open a fluorescence-based experiment, the software shows the fluorescence standard curve and a summary of the associated data for the first sample measurement, much like it appears during a measurement.

### Data Table—

Swipe left in any Spectral or Standard Curve Data screen to see the data table for the current experiment. The data table contains the measurement results for all samples in the experiment. The image below describes the available features.




### Menu

Select ⋮ at the top of any Spectral Data, Standard Curve, or Data Table screen to see the available menu options.

| | |
|---|---|
| Data Export & Report | Export or print opened experiment |
| Tags | Add or delete labels for selected experiment to make it easier to find (see Manage identifiers on the instrument) |
| Details | Open experiment details box which contains the experiment name, application type, date the experiment was created, number of measurements contains within the experiment, serial number of the instrument used, and information on the software used during the experiment |

# NanoDrop Ultra General Operations

These operations are available from any measurement screen or from the History.

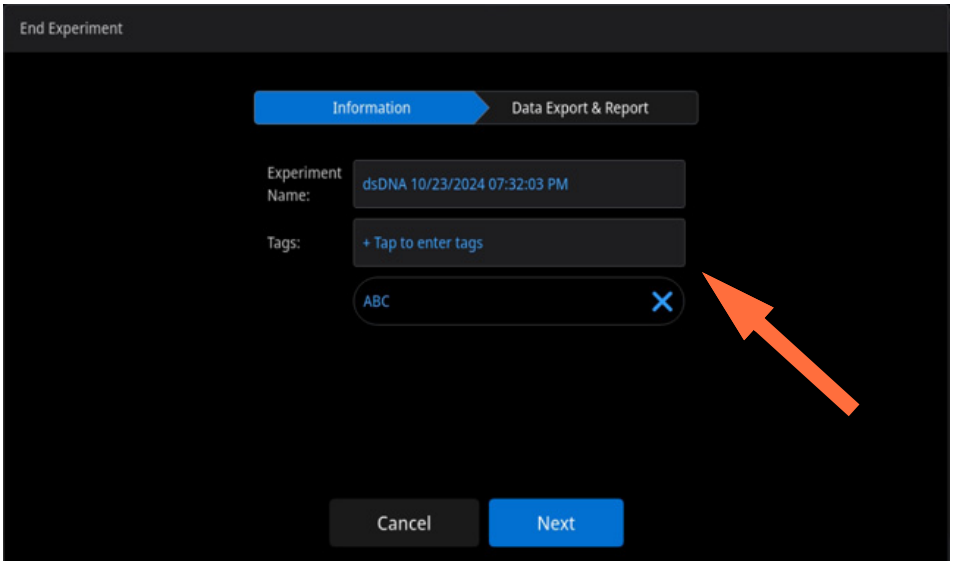
## Manage Identifiers

You can add one or more tags (i.e., labels or metadata tags) to an experiment to make the experiment easier to find. Labels can be added from the NanoDrop Ultra local control software running on the instrument, or from the NanoDrop Ultra PC control software installed on a personal computer.

Use the History to add labels to experiments, assign existing labels, view assigned labels and remove or delete labels on the instrument. You can filter the list of experiments in the History based on one or more user-defined labels.

### Label new experiment when you save it

- After the last sample has been measured, tap End Experiment
- In End Experiment window, tap on the field that says "Tap to enter tags." A keyboard will appear
- Enter user defined labels and tap **Done** to close the keyboard and add the tag




- Select **Next** to Export or Print
- Select **Finish** to close the experiment

### Label experiment in History

- From the home screen, select to open History
- Select to open the menu options of the experiment row and select **Tags**

- in Manage Tags window, tap on the field that says "Tap to enter tags." A keyboard will appear
- Enter user defined labels and tap **Done** to close the keyboard
- Select **Save** to confirm the changes and close the Manage Tags window. Add additional tags as needed

### View assigned labels for an experiment

- From the home screen, select  to open History
- The tag(s) will appear below the experiment name

### Find labeled experiments

- From the home screen, select  to open History
- Enter the tag name into the **Search** bar

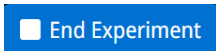
### Remove a label

- From home screen, select  to open History
- Select  to open the menu options of the experiment row and select **Tags**
- In Manage Tags window, select  for any tag you wish to delete
- Select **Save** to confirm the changes and close the Manage Tags window

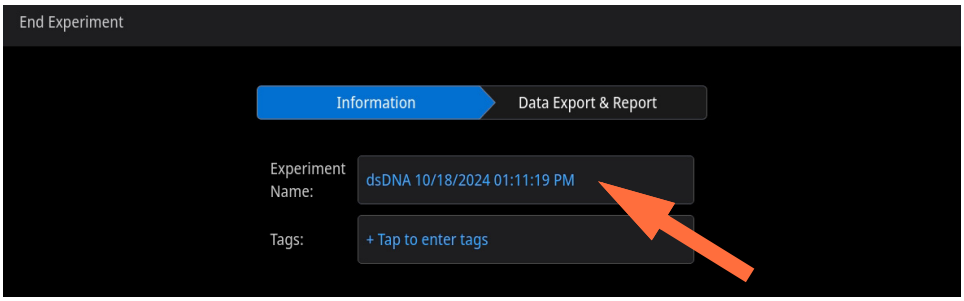
## Edit Experiment Name

You can edit the experiment name when you save the experiment or afterwards from the History.

### Edit experiment name at end of experiment

- When finished measuring samples, select 

- Enter a name for this group of measurements in the **Experiment Name** field




- Select **Next**
- Select **Continue** to export or print the experiment
- Select **Finish** to close the experiment

### Edit experiment name from History

- From home screen, tap  to open History
- Use Search feature to find experiment
- Double click or tap the experiment to open experiment select the **Experiment Name** field to display keyboard
- Enter new experiment name
- Tap **Done** key to close keyboard and save the new name

## Export Selected Experiments

You can export measurement data when you save the experiment or afterwards from the History.

**Note** Data exported during a save are still saved to a database (local or remote, depending on the Data Storage setting.

Measurement data can be exported in three formats:

- As comma-separated values (.csv) files containing one or more of the following:
  - whole spectrum data in columns
  - report data in columns
  - application name
  - serial number
- As tab-separated values (.tsv) files containing one or more of the following:
  - whole spectrum data in columns
  - report data in columns
  - application name
  - serial number
- As NanoDrop Ultra software (.ndu) files containing spectra and measurement results for each exported experiment

Use any spreadsheet or word processing application to open a CSV or TSV file. Here is an example of several sample measurement results in CSV format:

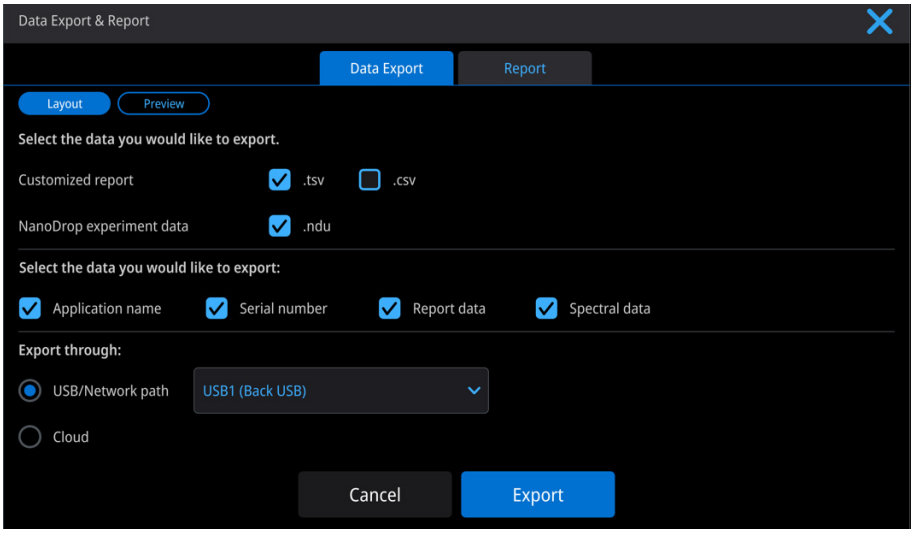
| | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Application: | Fluorometer | | | | | | | | |
| 2 | Serial Number: | NUI2400006 | | | | | | | | |
| 3 | Assay | Blue (470 nm) | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |
| 6 | # | Date | Sample Name | mg/mL | Original Conc. | Dilution Factor | RFU | Average RFU | # of Replicates | Excitation LED |
| 7 | 1 | 10/16/2024 9:01 | Reference | 0 | 0 | 1 | | 105.603 | 1 | Blue |
| 8 | 2 | 10/16/2024 9:02 | Standard 1 | 5 | 5 | 1 | | 1.742 | 1 | Blue |
| 9 | 3 | 10/16/2024 9:02 | Sample 1 | 4.122 | 4.122 | 1 | 19.978 | | | Blue |
| 10 | 4 | 10/16/2024 9:02 | Sample 2 | 3.992 | 3.992 | 1 | 22.678 | | | Blue |
| 11 | 5 | 10/16/2024 9:03 | Sample 3 | 3.87 | 3.87 | 1 | 25.206 | | | Blue |
| 12 | | | | | | | | | | |
| 13 | | | | | | | | | | |
| 14 | | | | | | | | | | |

**Note** The types of data exported are dependent upon the application used to measure the samples (nucleic acids in this example). For more information, see the application details.

Data can be exported to a USB device connected to any USB port on the local instrument (front, back-left or back-right), a connected Cloud account, or to a network location. If you select multiple experiments for export, each exported experiment has a corresponding file. The filenames are the same as the experiment names. The files are stored in a folder named “NanodropOne” followed by the instrument serial number. (Use System Status to view your instrument serial number.)

### Export data at end of experiment

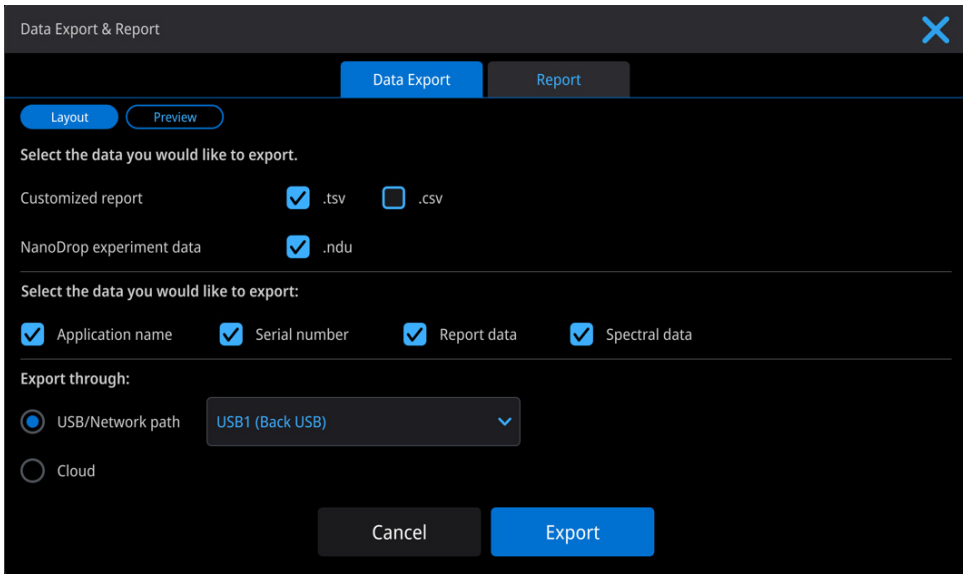
- When finished measuring samples, select End Experiment
- From End Experiment window, enter Tags and edit experiment name if desired then select **Next** to move to the Data Export & Report tab within the End Experiment window
- Select **Continue** to open the Data Export & Report window
- Select one or more export file types and one or more customized report options



- Designate the export location
- Select the **Preview** tab to see what the exported file will look like including any customization that was selected. This feature will not work for the .ndu export file type
- Select **Export**
- After "Export Completed" message, select **OK**

### Export data from History

- From home screen, tap  to open History
- Select the checkbox for the experiment(s) you want to export. You can use the Search feature to find experiment
- Select **Export**
- Select one or more export file types and one or more customized report options
- Designate the export location




- Select **Export**
- After "Export Completed" message, select **OK**

## Delete Selected Measurements

You can delete selected sample measurements from any experiment, or all the measurements in the database.

**Note** Deleted data can only be recovered if a database back up file containing that data was saved prior to the deletion.

### Delete data from any measurement screen

- Press and hold sample row to open Sample Details window
- Select 
- Select **Yes** to confirm the deletion of the sample measurement

### Delete data from History

- From home screen, tap  to open History
- Select the checkbox for the experiment(s) you want to delete. You can use the Search feature to find experiment
- Select 
- Select **Yes** to confirm the deletion of the experiment and all sample measurements within that experiment

## Print Selected Measurements

Connect a compatible printer to the instrument to quickly print measurement results, including spectral data, standard curves, data tables, sample details and diagnostic results. You can print to a USB printer (label or full service) or to a remote printer through an Ethernet connection or wireless network.

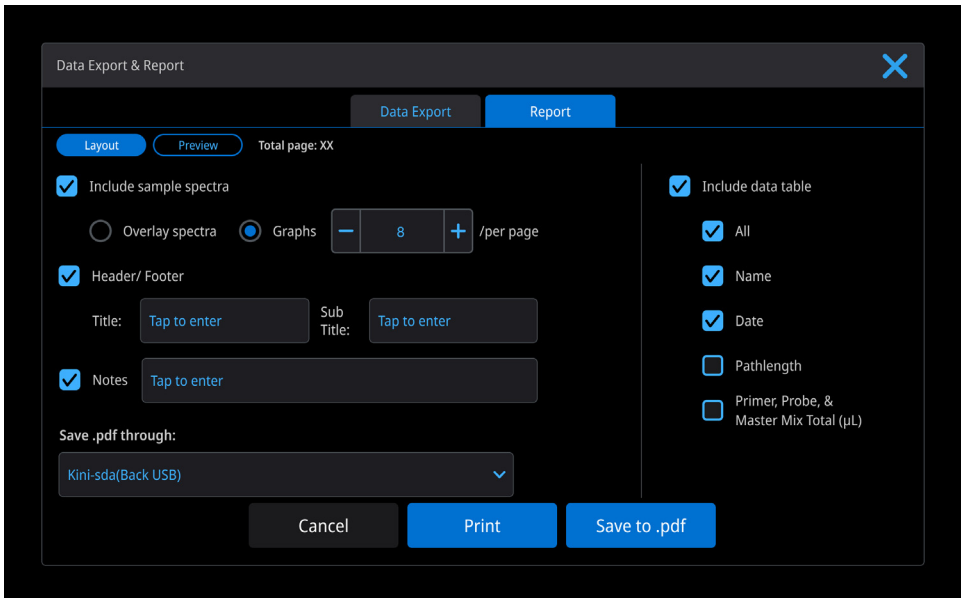
**Note**

- A wireless printer or the device it is connected to must be available on the same wireless network as the instrument. The wireless printer must also have its wireless function enabled.
- Full service printer options are not available if you have a label printer connected. Disconnect the label printer to access the full service printer options.

### Print data at end of experiment

- When finished measuring samples, select 

- From End Experiment window, enter Tags and edit experiment name if desired then select **Next** to move to the Data Export & Report tab within the End Experiment window
- Select **Continue** to open the Data Export & Report window
- Select the **Report** tab
- Select one or more customized report options

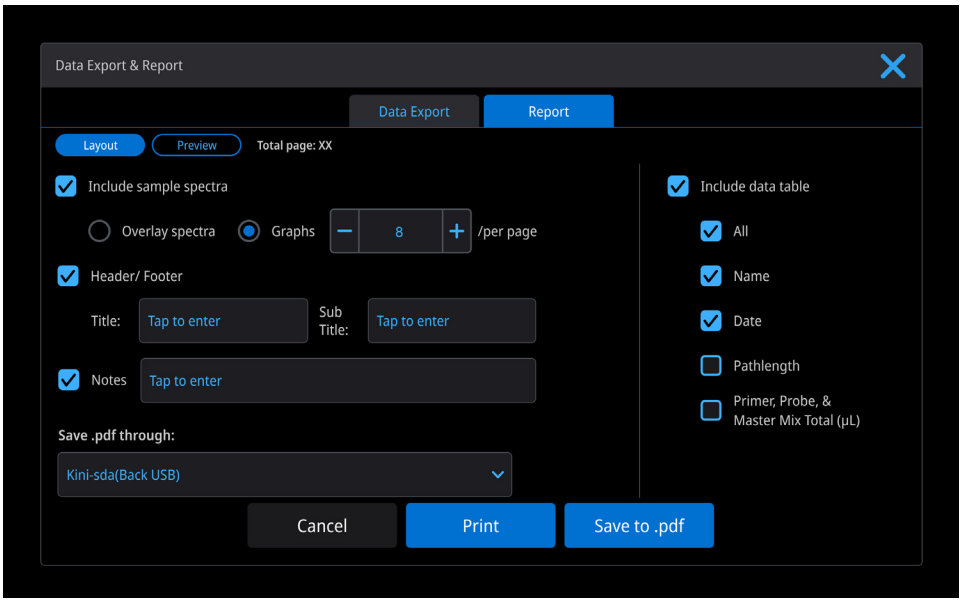



- Designate the print to PDF location if necessary
- Select the **Preview** tab to see what the printed report will look like including any customization that was selected
- Select **Print** to select an available printer to print to or select **Save to .pdf** to export a .PDF file to the specified location
- After "Export Completed" message, select **OK**

If a label printer is connected to the instrument, the software prints one label for each selected measurement. If a full service printer is connected, the selected measurement screen is printed for each selected measurement.

### Print data from History

- From home screen, select  to open History
- Select the checkbox for the experiment(s) you want to print. You can use the Search feature to find experiment
- Double click or tap the experiment name to open the experiment
- Select  at the top of any Spectral Data, Standard Curve, or Data Table screen to see the available menu options and select **Data Export & Report**
- Select the **Report** tab
- Select one or more customized report options

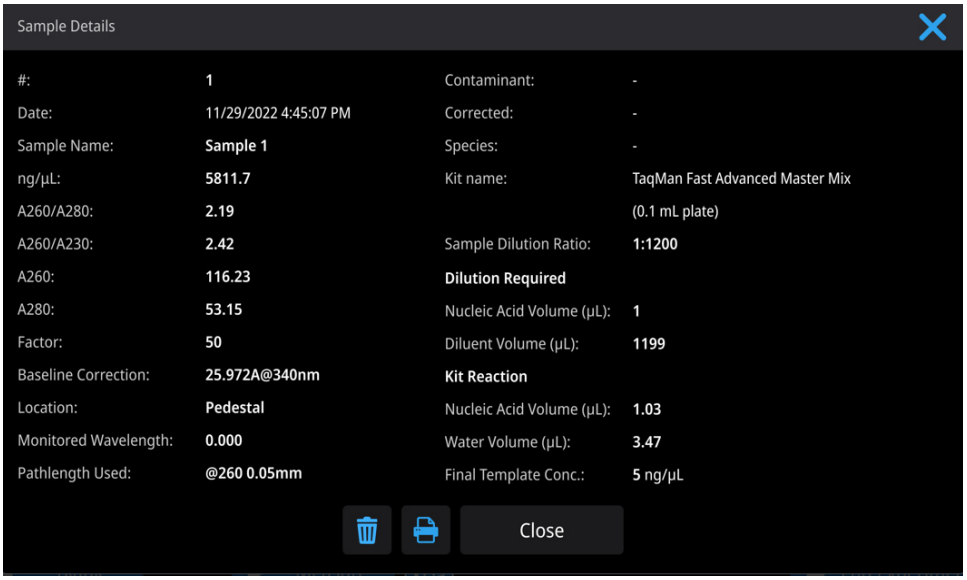



– Designate the print to PDF location if necessary
– Select the **Preview** tab to see what the printed report will look like including any customization that was selected
– Select **Print** to print to a connected printer or select **Save to .pdf** to export a .PDF file to the specified location
– After "Export Completed" message, select **OK**

If a label printer is connected to the instrument, the software prints one label for each selected measurement. If a full service printer is connected, the selected measurement screen is printed for each selected measurement.

### Print sample details

– From the spectral data, standard curve, or data table in any measurement screen or from History, press and hold sample row to open Sample Details box




– Select 

– Select an available printer from the down-down menu and select Print

If a label printer is connected to the instrument, the software prints a label for the selected measurement. If a full service printer is connected, the selected sample details screen is printed

## LIMS API Integration

The REST API allows for efficient data exchange, including the submission, retrieval, and updating of data between the instrument and your LIMS. For more information on how to utilize the REST API, please refer to the NanoDrop Ultra API Reference Manual.

# Acclaro Sample Intelligence

The Thermo Scientific™ Acclaro™ Sample Intelligence technology built into the NanoDrop Ultra instruments provides these exclusive features to help you assess sample integrity:

- contaminant analysis to help qualify a sample before use in downstream applications
- on-demand technical support for measurements that are atypical or very low concentration

Use these embedded resources to quickly troubleshoot possible problem measurements and make informed decisions on whether to use, re-purify or take other actions with an atypical sample result. The Sample Intelligence feature also serves as a resource for further study and a learning tool for new or novice users.

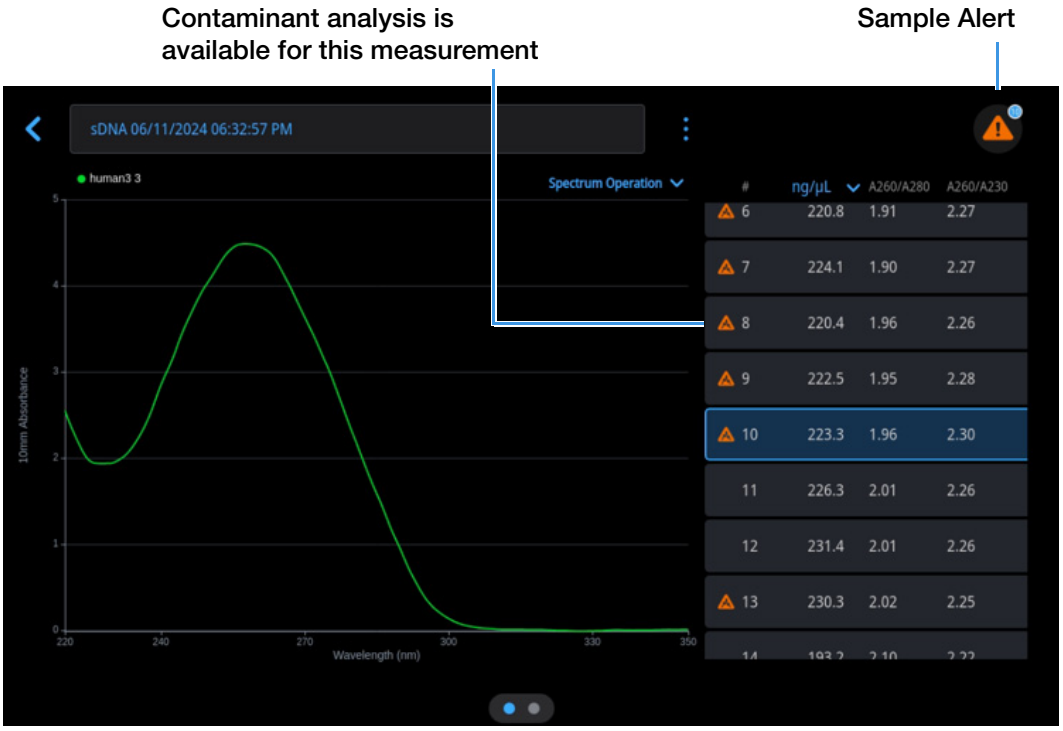
## Activate Detection

From the measurement setup screen, select the activate contaminant detection option and choose between Mammal, Plant, and Bacteria.

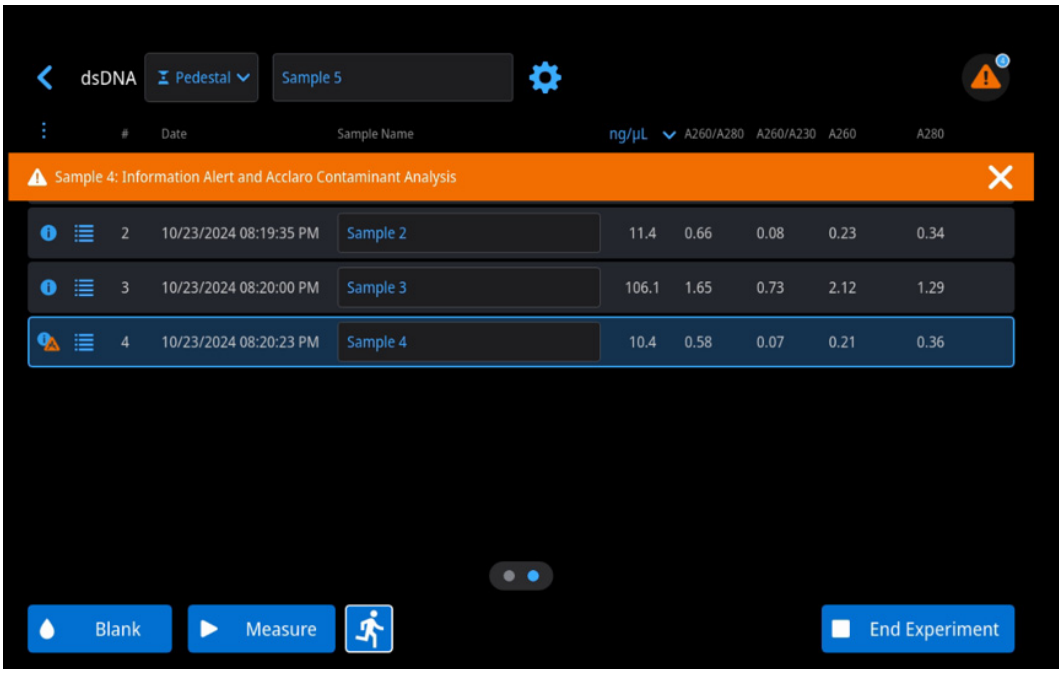

When enabled, RNA Contaminant Detection/DNA Contaminant detection will apply mathematical models to predict the amount of RNA contaminant in dsDNA or dsDNA in RNA for the selected species. These models are specific to the source of the nucleic acid. For example, if mammal is selected, the mathematical model will be representative of all mammalian sources of nucleic acid. If you are measuring nucleic acid from a source for which we do not have mathematical model, leave the selections unchecked.

# View Acclaro Sample Intelligence Information

Measurements that include a contaminant analysis or technical information are flagged automatically (see examples below). Select the icon to review the associated data or information. The alert icon and banner will also appear at the top of the screen.




The icons appear next to the measurement results (see above), and in the data table, as well as in History (see below).



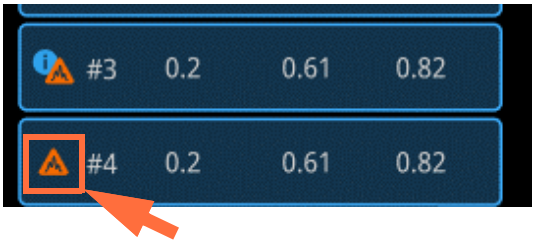

The icons are active in all three places; the information remains with the data indefinitely, even after it has been exported.

## Contaminant Analysis

For the dsDNA, RNA and Protein A280 applications, the NanoDrop Ultra software automatically initiates a spectral analysis for several known contaminants during the measurement. Examples of known contaminants include:

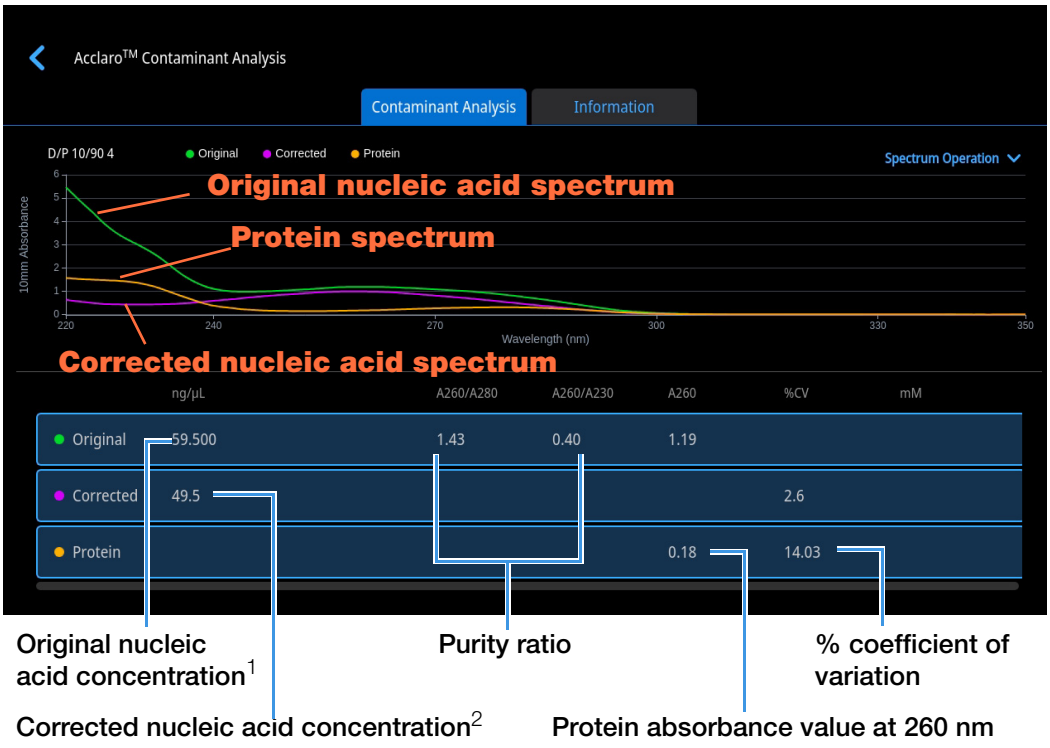
- For dsDNA and RNA measurements:
  - in the analysis region: protein and phenol
  - detects presence of guanidine HCl and guanidinium isothiocyanate
  - detects species-specific dsDNA contamination in the RNA application and detects species-specific RNA contamination is in the dsDNA application
- For protein measurements:
  - in the analysis region: nucleic acids and phenol

If contaminants are identified in a sample, the “Contaminant Analysis” icon appears to the left of the measurement results.




Tap the icon to view the contaminant analysis and associated information.

The following is an example of results from a nucleic acid contaminant analysis that contains enough protein contaminant to affect the measurement results.




[1]Based on total sample absorbance (sample plus contaminant)

[2]Based on corrected sample absorbance (sample minus contaminant)

Since proteins absorb light near the analysis wavelengths for nucleic acid (230 nm, 260 nm, and 280 nm), the presence of protein in the nucleic acid sample shown above has pushed the A260/A280 and A260/A230 ratios out of range and caused the reported nucleic acid concentration to be higher than the real value. The software identifies the impurity (protein), and reports the following:

- baseline-corrected absorbance due to protein (0.18) at the analysis wavelength (260 nm)

- % coefficient of variation for the measurement result (uncertainty x 100/measurement result = 14.03%; a high %CV indicates the measurement result is close to the instrument detection limit or there is an interfering component)
- original nucleic acid concentration (59.5 ng/µL), which is based on the total baseline-corrected absorbance (sample plus contaminant) at the analysis wavelength
- corrected nucleic acid concentration (49.5 ng/µL), which is based on the corrected absorbance (sample minus contaminant) at the analysis wavelength

## Theory behind contaminant analysis

UV and UV-visible absorbance measurements are used to quantify nucleic acid and protein samples at 260 nm and 280 nm, respectively. The analysis is based on the fact that the total absorbance of a mixture solution at a given wavelength is the sum of the absorbance values of each component in the mixture.

An ongoing challenge of this method is that a number of materials used in the extraction process can absorb in various regions across the spectrum. When these contaminants are present in a sample, they can interfere with the analysis by artificially inflating the absorbance at the wavelength of interest, which causes the analyte concentration to be overestimated.

Traditionally, purity ratios are used to detect the presence of contaminants that could affect downstream applications. However, purity ratios do not always provide a complete picture of possible contamination. When a purity ratio falls outside the expected range, the spectral profile is often examined qualitatively.

Our Acclaro technology applies a quantitative approach to contaminant analysis. Through sophisticated mathematical algorithms, Acclaro analyzes the spectral data to identify probable contaminants in a sample and removes any contribution due to the contaminant from the sample result. This results in a more accurate concentration value of the analyte of interest and a more quantitative analysis of the level of contamination.

Since the spectrum of a pure compound is unique to that compound, a mixture spectrum of mostly known materials that have few interactions can be mathematically broken down into its component spectra and the components identified. The contaminant analysis algorithm uses a narrow spectral region (220-285 nm) around the analysis wavelength (260 nm for nucleic acids, 280 nm for proteins) to determine any absorbance contribution from possible known contaminants (protein or nucleic acid, and phenol) that absorb in that region. The entire spectrum is analyzed to determine the presence of other possible contaminants such as guanidine HCl and/or guanidinium isothiocyanate, which are common reagents used for nucleic acid purification.

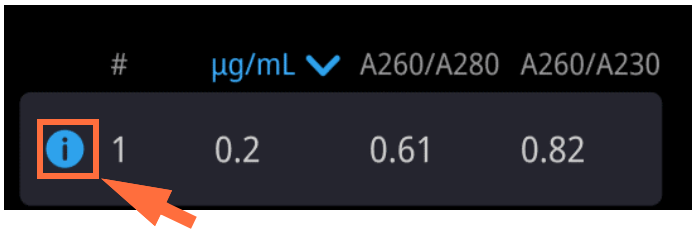
**Note** Achieving consistent, high quality contaminant analysis results is dependent on the quality of the measured sample spectra, which is dependent on the maintenance status of the instrument. For more information, see Maintenance Schedule.

## On-Demand Technical Support

For the dsDNA and Protein A280 applications, the NanoDrop Ultra software monitors all sample measurements for the presence of contaminants or other anomalies that may affect the measurement. Examples of monitored characteristics include:

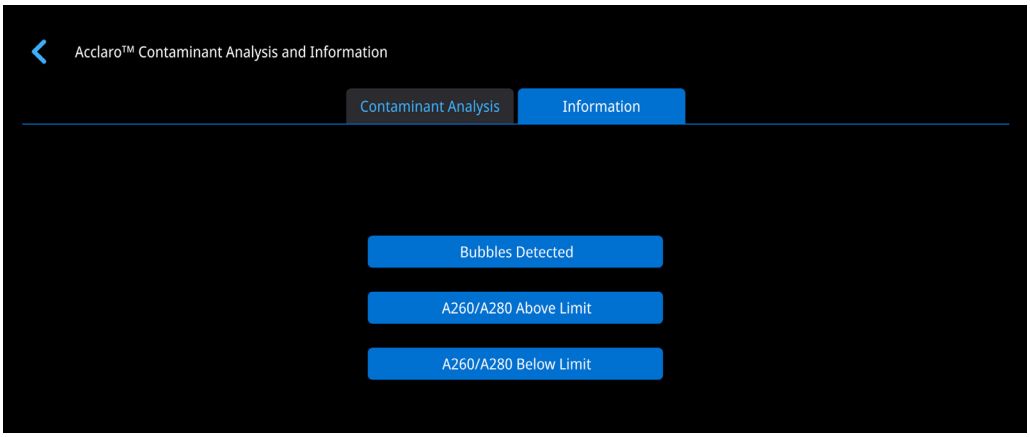
- Absorbance ratios, which indicate the presence of compounds that may interfere with sample measurements (also referred to as “purity ratios”). For more information, see What is a Purity Ratio?.
- Bubble check, which looks for bubbles or other reflective materials in a sample or blank.

If technical information is available, the “information” icon appears to the left of the measurement results. The alert icon and banner will also appear at the top of the screen.

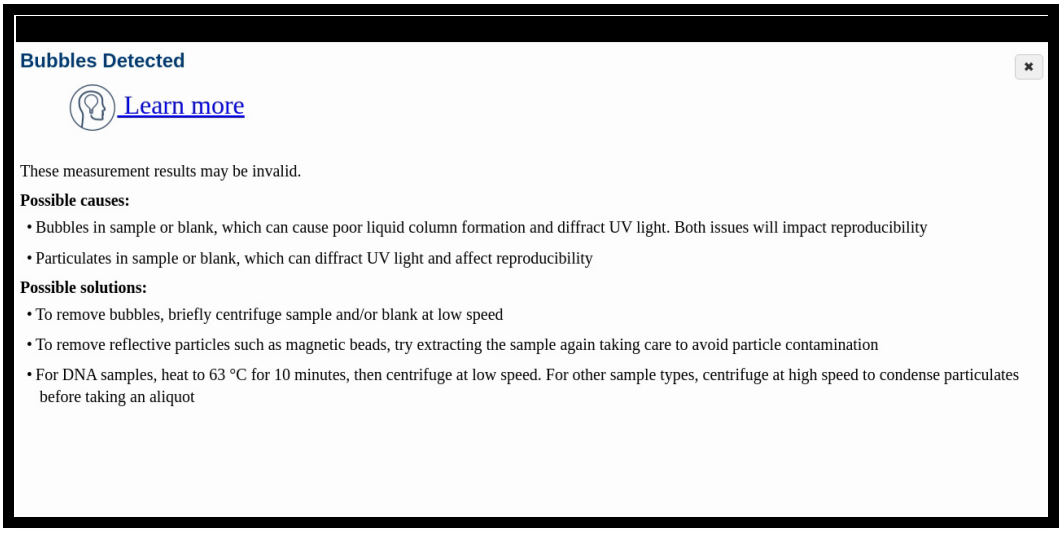



Tap the icon to view the information.

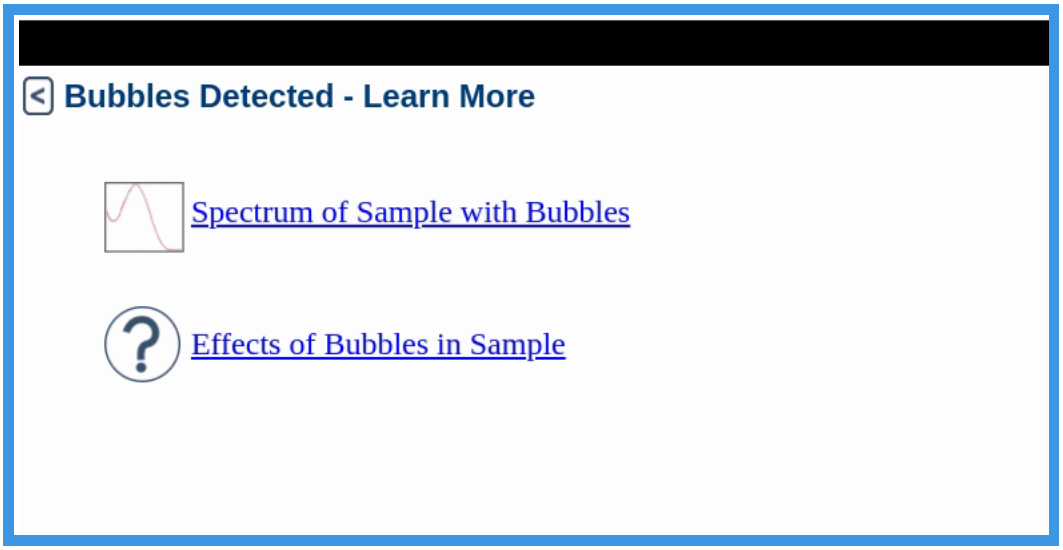
Here are results from a nucleic acid analysis for which two measured purity ratios are either above or below the expected value and the sample contained enough bubbles to possibly affect the measurement results.



Tap an information button for more information. Here is the information provided for the bubble error:




Tap **Learn More** for the next level of information.



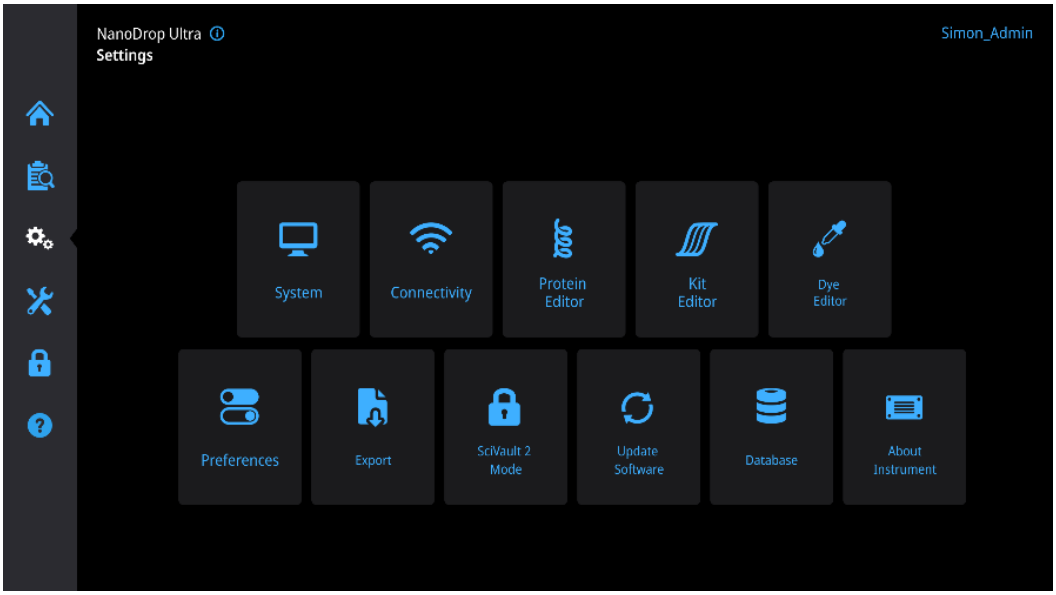

## Alarm Notifications

The NanoDrop Ultra software uses an embedded image sensor to monitor all measurements for conditions (such as a broken liquid column) that are likely to invalidate the measurement results.

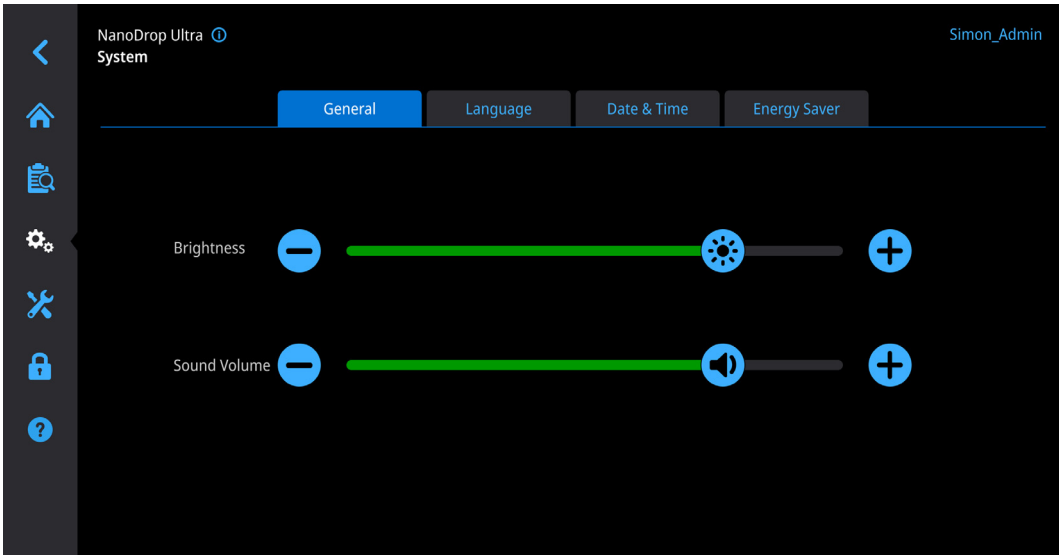
After an invalid-result, the Alarm icon is displayed and the measurement is stopped. See Troubleshooting for more information.

# Instrument Settings

From the home screen, select . Available options include System Settings, Connectivity Settings, Protein Editor, Kit Editor, Dye Editor, Preferences, Export Settings, SciVault 2 Mode, Update Software, Database Settings, and About Instrument.




## System Settings



**These options are available**:

| | |
|---|---|
| **General** | Increase or decrease screen brightness and volume on instrument by using the respective sliders |
| **Language** | Select language for displaying NanoDrop Ultra software and for any connected input device such as a keyboard, mouse or barcode scanner<br><br>**Notice**: Changing the language requires a software restart. |
| **Date and Time** | **Automatic Time Zone & Time**: synchronize instrument time zone, time, and date with available network<br><br>**Select Time Zone**: manually select instrument time zone (this option is disabled when Automatic Time Zone & Time is selected)<br><br>**Set Time & Date**: manually set instrument time and date (this option is disabled when Automatic Time & Date is selected)<br><br>**Choose Date Format**: choose an available date forma<br><br>**Choose Time Format**: choose an available time format |
| **Energy Saver** | The touchscreen will turn off and a screen saver will be displayed when the instrument has been idle for the selected time. To reactivate the instrument, tap the touch screen. |

## Connectivity

These options are available:

| | |
|---|---|
| **Wi-Fi** | Use this tab to enable and specify a Wi-Fi connection for the instrument. The NanoDrop Ultra Wi-Fi and Bluetooth Dongle must be inserted into the instrument, or this feature will be unavailable. |
| **Bluetooth** | Use this tab to enable and specify a Bluetooth connection for the instrument. The NanoDrop Ultra Wi-Fi and Bluetooth Dongle must be inserted into the instrument, or this feature will be unavailable. |
| **Cloud** | Use this tab to connect to the Thermo Fisher Connect Platform which enables direct data export to Thermo Fisher Cloud Connect, Google Drive, or Microsoft OneDrive. Some Cloud platforms may only be available in select global locations. |

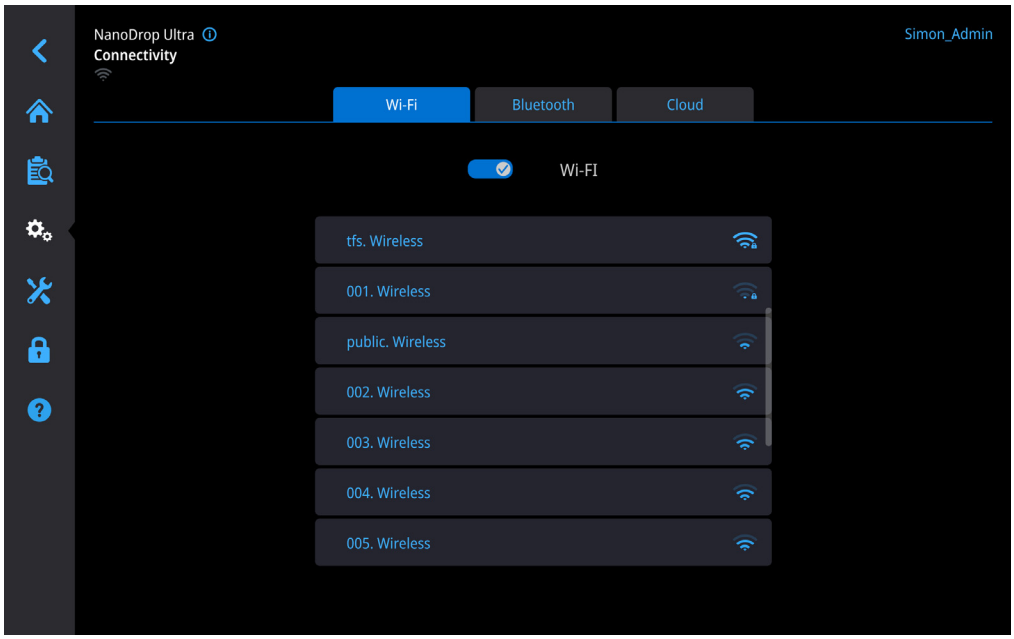
## Wi-Fi

Use this tab to enable and specify a Wi-Fi connection for the instrument. The NanoDrop Ultra Wi-Fi and Bluetooth Dongle must be inserted into the instrument, or this feature will be unavailable.

### Setup Wi-Fi network on the instrument

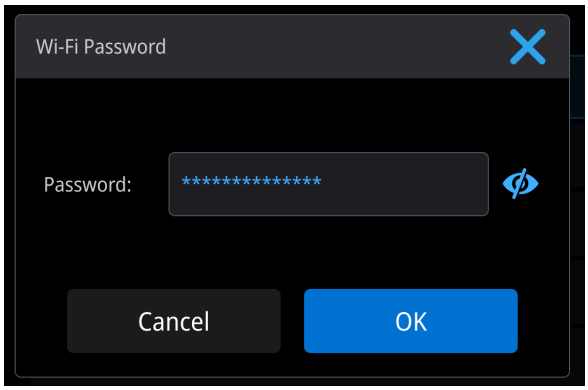
1. From the instrument home screen, select  (**Settings**).
2. Select **Connectivity**, then select the **Wi-Fi** tab at the top.
3. Use the slide toggle to enable Wi-Fi connectivity (turns blue with a checkmark and software automatically searches for any available Wi-Fi network)

   If no Wi-Fi networks are found, after a few seconds the message "No nearby Wi-Fi networks were found" is displayed
4. The NanoDrop Ultra instrument will continuously refresh the list of available networks.
5. The Wi-Fi name should appear in the provided list.

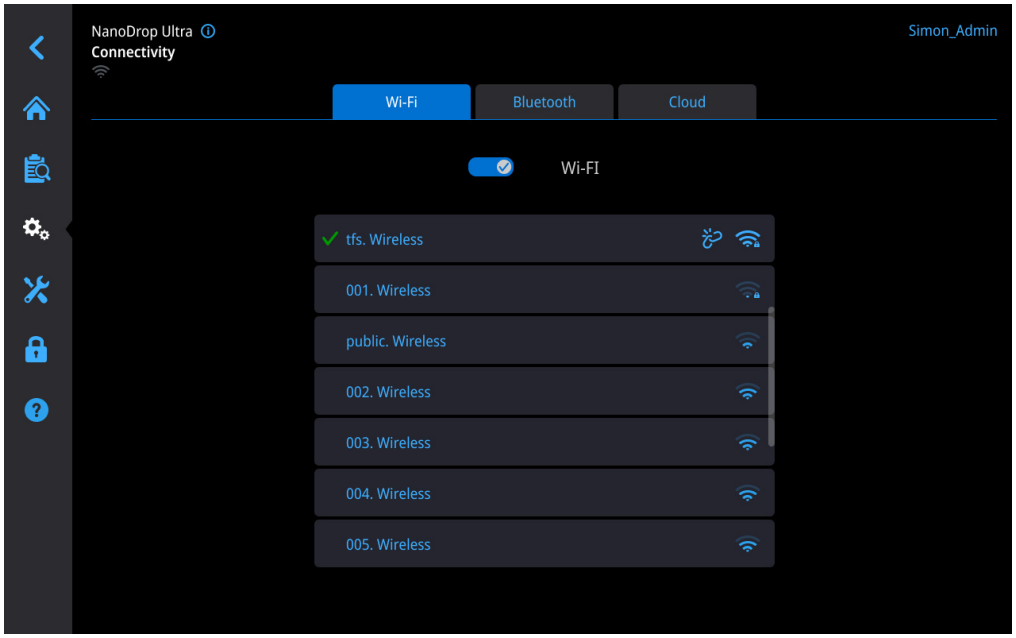



6. To connect to a Wi-Fi network, tap its name in Available Network list.

7. If the Wi-Fi network is password protected, a window will pop-up requesting entry of the Wi-Fi password. Enter the password and select **OK**.




8. A green checkmark will appear next to the name if the pairing was successful.




9. When Wi-Fi is enabled and connected, a Wi-Fi icon will appear at the top of the screen.
10. Select back to return to the Settings screen.

### Disconnect Wi-Fi network

To disconnect from a connected Wi-Fi network, select the disconnect icon and then select **Forget** to confirm.

## Bluetooth

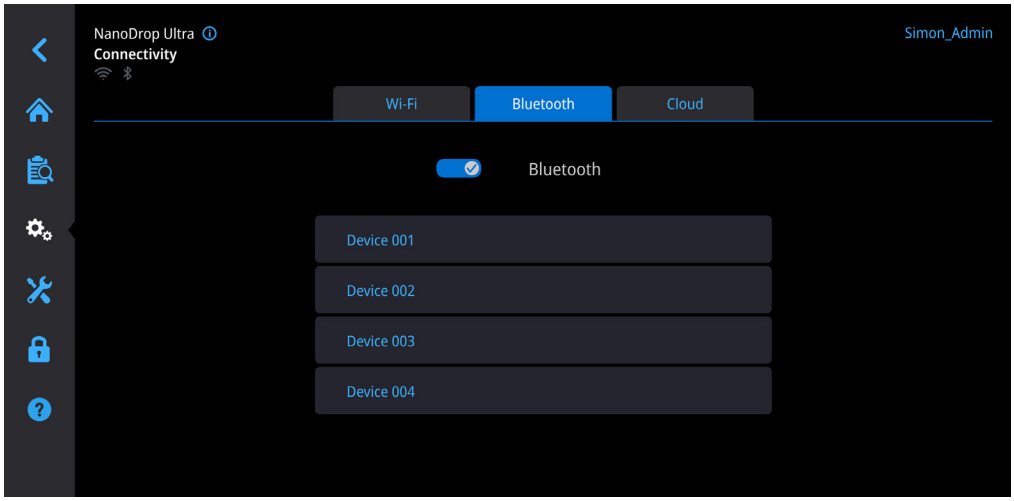
Use this tab to enable and specify a Bluetooth connection for the instrument. The NanoDrop Ultra Wi-Fi and Bluetooth Dongle must be inserted into the instrument, or this feature will be unavailable.

**Note** Make sure the device is labeled "Bluetooth" and not just "wireless." All Bluetooth devices are wireless but not all wireless devices will run with Bluetooth.

### Set up Bluetooth connections on the instrument

1. From the instrument home screen, select (**Settings**).
2. Select **Connectivity**, then select the **Bluetooth** tab at the top.
3. Use the slide toggle to enable Bluetooth connectivity (turns blue with a checkmark and software automatically searches for any available Bluetooth input devices)

   If no Bluetooth devices are found, after a few seconds the message "No nearby Bluetooth devices were found" is displayed
4. To add a Bluetooth device, follow the manufacturer instructions to pair the device (for example, you may need to hold down a button). The NanoDrop Ultra instrument will continuously refresh the list of available devices.
5. The device name should then appear in the provided list.




6. To pair a device, tap its name in Available Devices list, a green checkmark will appear next to the name if the pairing was successful.
7. When Bluetooth is enabled and connected, a Bluetooth icon will appear at the top of the screen.

8. Repeat steps above to add another Bluetooth device or select back  to return to the Settings screen.

**Note** If your Bluetooth device does not pair, restart the device and then repeat the steps above to pair it with the instrument (you may also try turning Bluetooth off and back on). After a device is paired, it remains paired even after the instrument is restarted.

### Disconnect Bluetooth device

To disconnect from a connected Bluetooth device, select the disconnect icon  and then select **Forget** to confirm.

**Note**

- If no Bluetooth device is selected for input, the instrument relies on the integrated touchscreen keyboard for input.
- To select the device again, follow the steps above and select the device's Use for Input checkbox.

## Cloud

Use this tab to connect to the Thermo Fisher Connect Platform which enables direct data export to Thermo Fisher Connect, Google Drive, or Microsoft OneDrive.

### Create a Thermo Fisher Scientific Cloud Connect Account

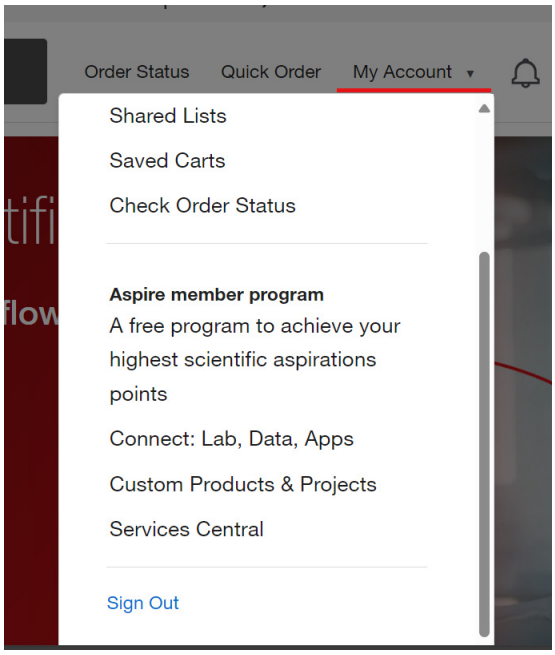
During the initial Cloud setup, the instrument needs to be connected to the Thermo Fisher Cloud Connect Platform. A Thermo Fisher account must be used during this process and requires a one-time setup.

1. From a PC browse to www.thermofisher.com/cloud
2. Select **Sign in** which will display a dropdown menu
3. Select either **Sign in** again if you already own a Thermo Fisher account or **Create Account** if you do not already have one and complete the additional steps to create a free account.

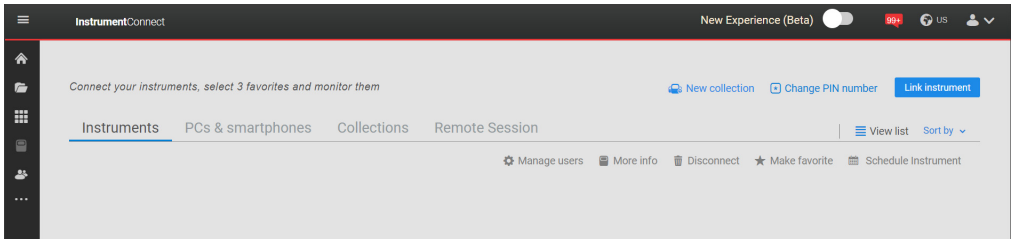
### Create a Thermo Fisher Cloud Connect PIN

1. After signing into your Thermo Fisher account in your preferred browser, select **My Account**

2. Select **Connect: Lab, Data, Apps**.




3. Select the  icon on the left panel to open the Instrument Connect page
4. Select **Change PIN number** towards the upper right of the Instrument Connect page

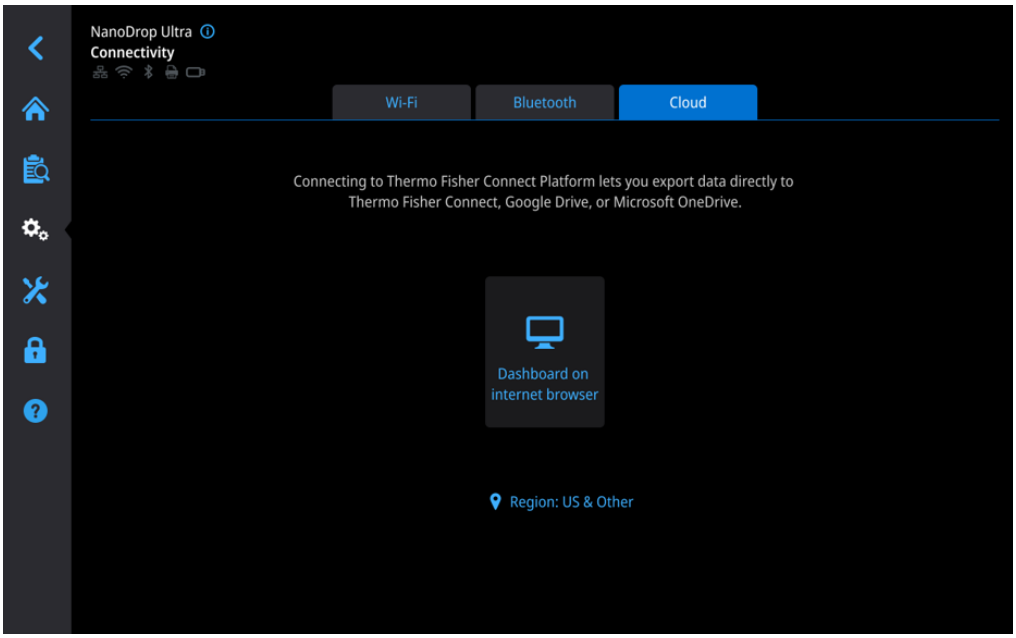



5. Enter a unique PIN number and save this information as you will need it later

### Connect to a Thermo Fisher Cloud Account

1. From the instrument home screen, select  (Settings).

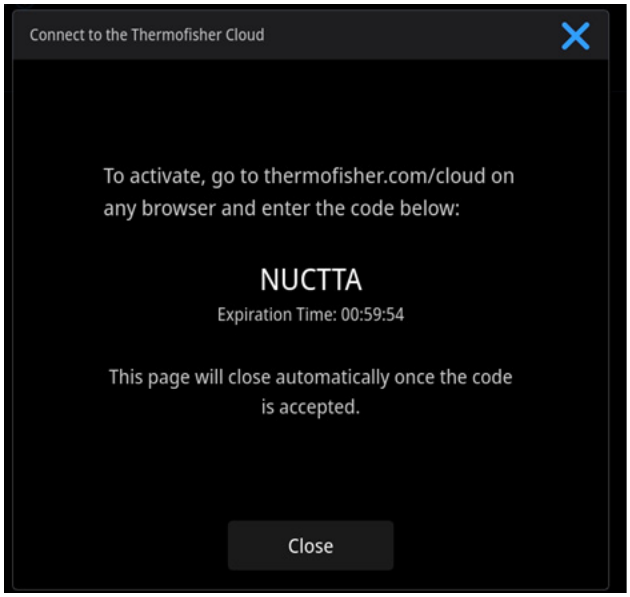
2. Select **Connectivity**, then select the **Cloud** tab at the top.



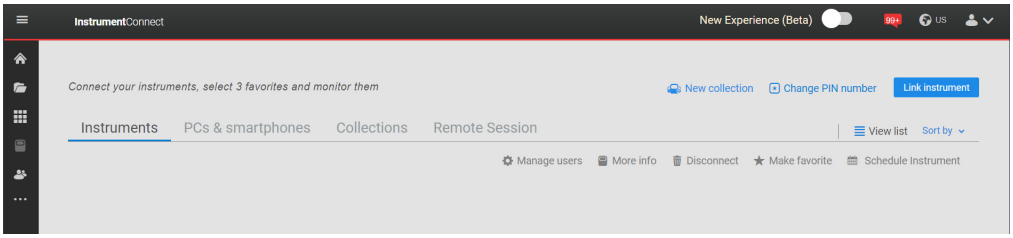

3. Ensure the appropriate Region to selected, the options are either US & Other or China.

**Note** When using China as a region, users will not be able to export to a Google Drive due to country limitations.

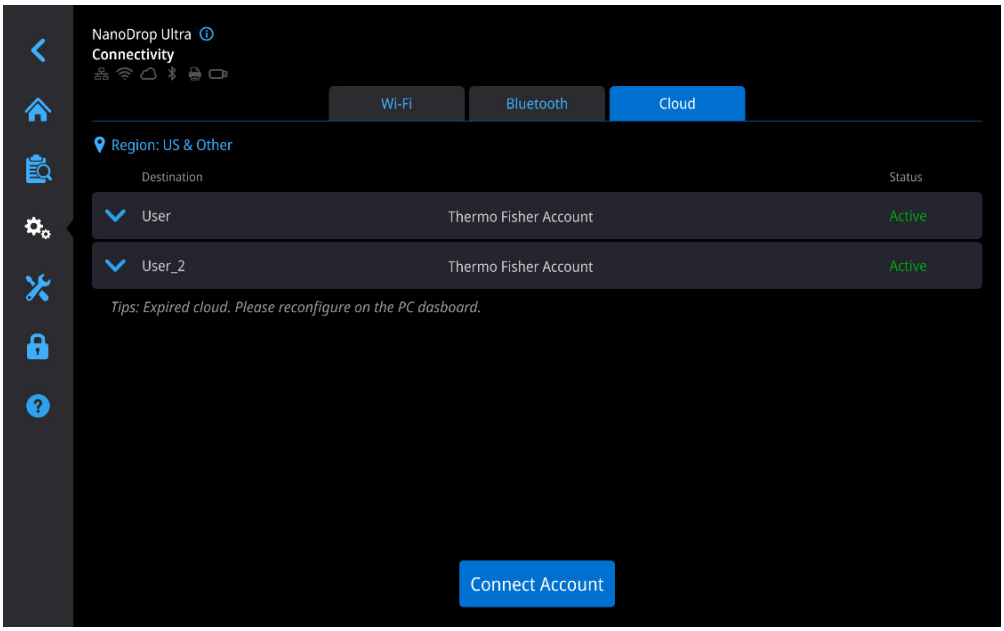
4. Select Dashboard on Internet Browser to display a unique linking code for the instrument.



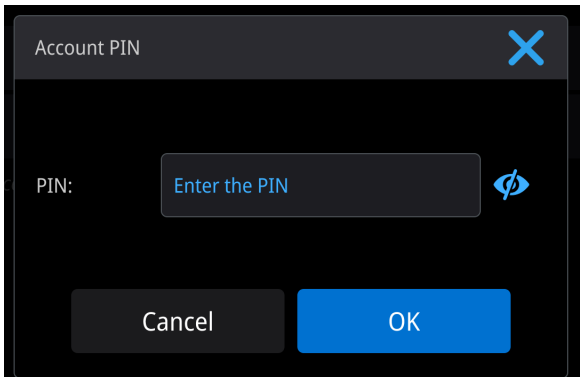
5. From a PC with internet access browse to www.thermofisher.com/cloud.
6. Sign into your Thermo Fisher account in your preferred web browser on a PC with internet access.
7. Select **Connect**: **Lab, Data, Apps**
8. Select the  icon on the left panel to open the Instrument Connect page



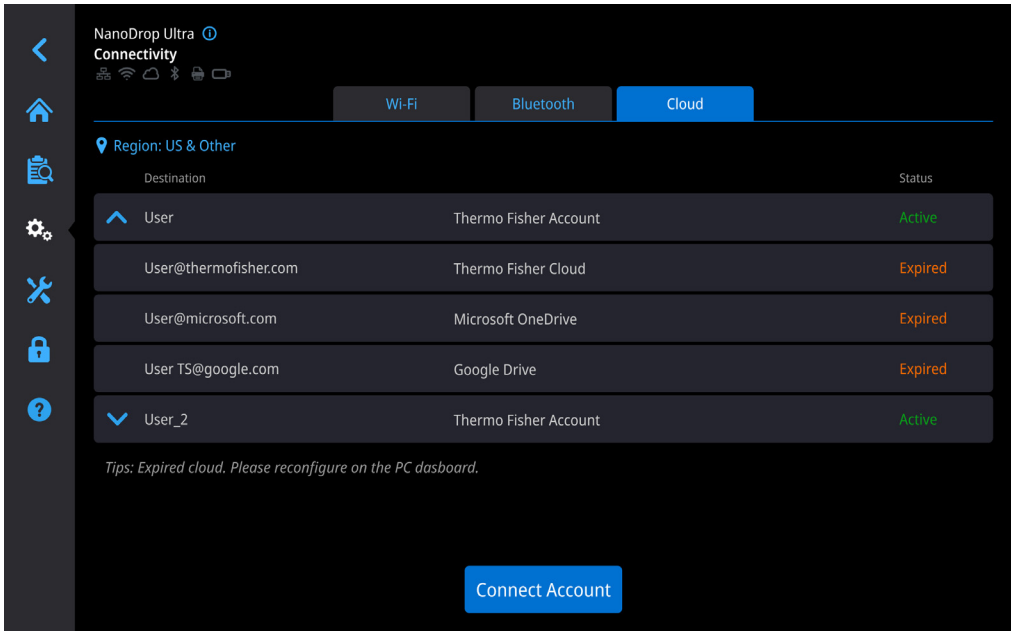

9. Select **Link instrument** and enter the unique linking code provided by the instrument
10. Select **Link**
11. The connected Thermo Fisher Scientific account will now appear in the Settings > Connectivity > Cloud tab with the Activated status.



12. Use  to display any Cloud accounts that have been associated with the Thermo Fisher Scientific account.




13. The Thermo Fisher Cloud Connect PIN will be requested, enter and select **OK**.




14. Select the Connect Account option and follow steps 5-10 to add additional Thermo Cloud Connect accounts.

### Connect to a Google Drive or Microsoft OneDrive Account

Visit https://www.thermofisher.com/cloud to connect a Google Drive or Microsoft OneDrive account to your Thermo Fisher Cloud Connect Account. Once connected, the additional Cloud options will appear in the software.

## Protein Editor

See “Protein editor” on page 95 for information on using the protein editor

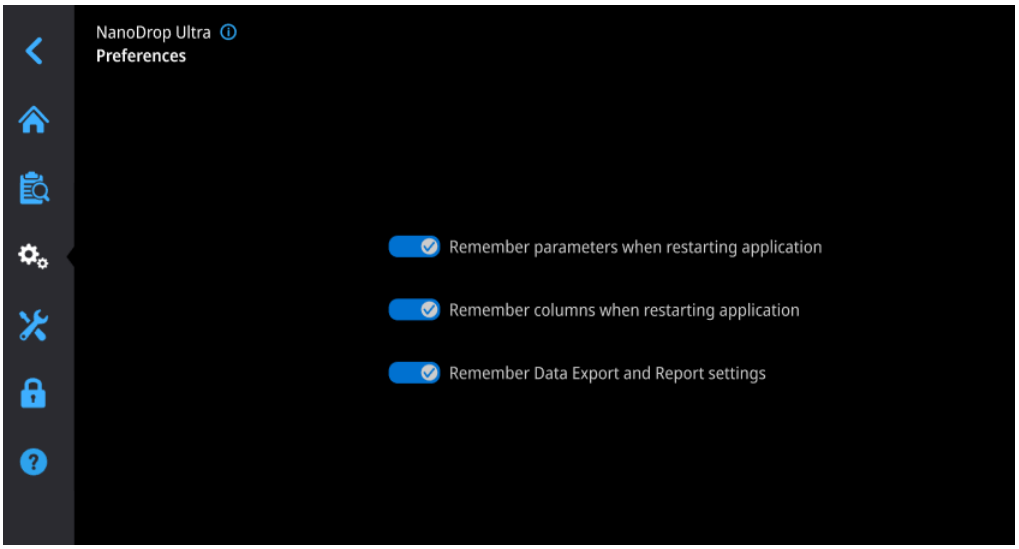
## Kit Editor

See Kit editor on page 44 for information on using the kit editor.

## Dye Editor

See Dye/chromophore editor on page 57 for information on using the dye editor.

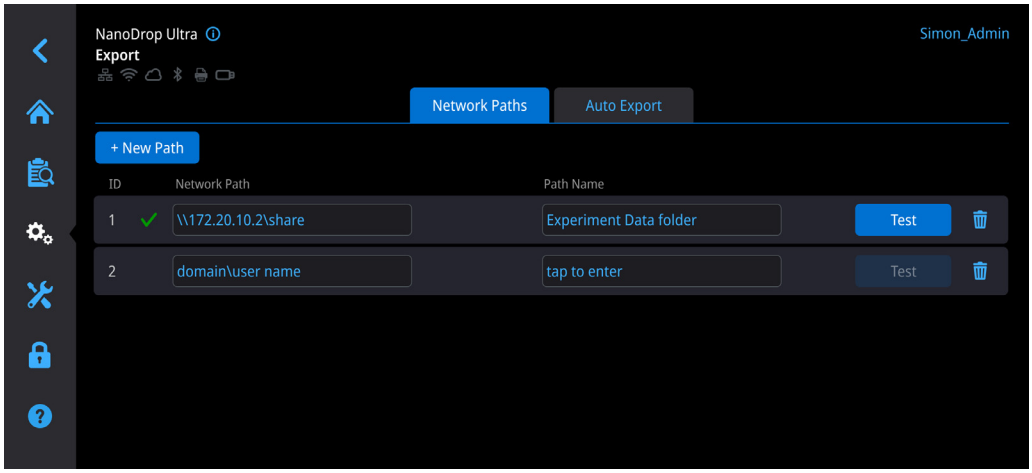
## Preferences




Set your preferences for remembering certain settings when restarting the application.

## Export Setup

These options are available:



## Network Paths

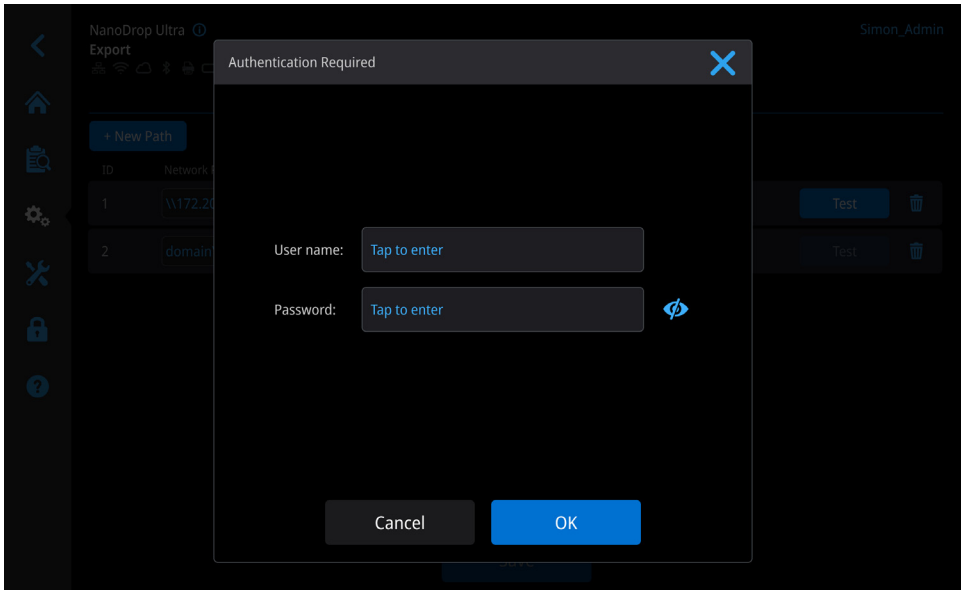
Use this tab to specify one or more network paths for exporting acquired data when the instrument is connected to a network (connection can be wired or wireless). Network paths defined here will appear in the USB/Network path drop-down menu when exporting data, from both the History and the End Experiment box after you complete a measurement.

### Add Network path

1. Select + New Path to add a new entry box for a network path each entry will have a unique ID.
2. Enter the Network Path in the following format: \\IP Address of Server or Server name\Name of the share drive\Name of folder on share drive. (from local control, tap field to display keyboard, tap **Done** key to close keyboard).
3. Enter a unique Path Name which will appear in the USB/Network path drop-down menu when exporting data.

### Test Network path

1. After all information has been input, select Test to confirm connection and access to the network location.
2. Enter a User name and Password for the network and are trying to connect to and select **OK**.



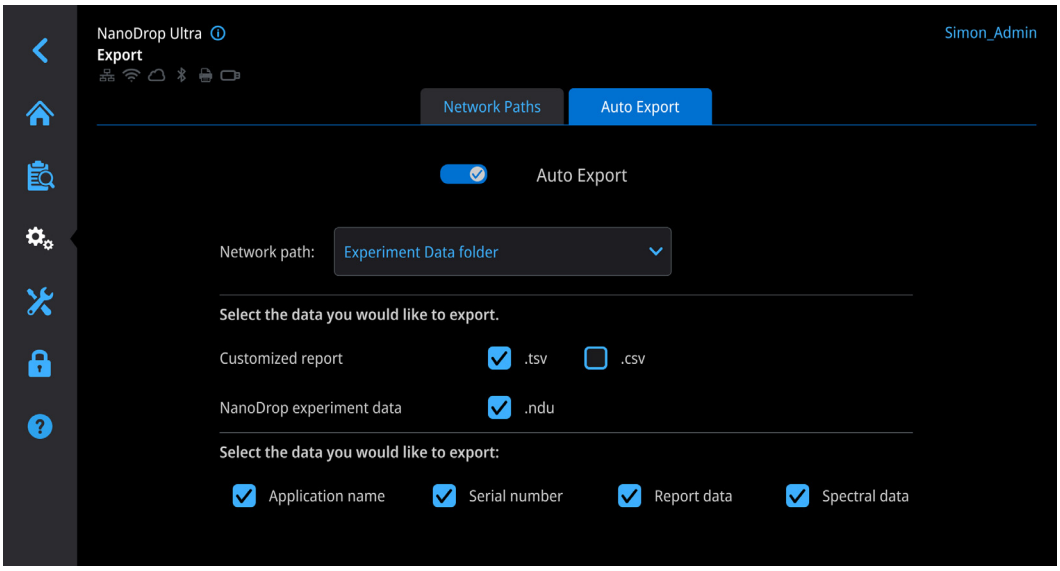

### Edit or Delete Network path

1. The Network Path and Path Name can be edited at any time by selecting each entry box and making any necessary changes.

2. Select to the right of the row containing the Network Path to delete it.

### Auto Export

Select a file path for exported files and enable/disable Auto-export at the end of experiments. A Network Path must be setup prior to enabling Auto Export. Select the desired network path location, file type(s) to export, and customizable settings. When enabled the software will save a copy of the experiment in the provided file type(s) to the location specified.




## SciVault 2 Activation

Setup the optional add-on Thermo Scientific™ SciVault™ 2 software. This companion software allows users to operate their NanoDrop Ultra instrument in a manner that complies with US FDA 21 CFR Part 11. When purchased, the SciVault 2 software and activation key is shipped to you on a USB stick and integrates directly into the NanoDrop Ultra software user interface.

See the SciVault 2 User Guide for information on activating and installing SciVault 2 software.

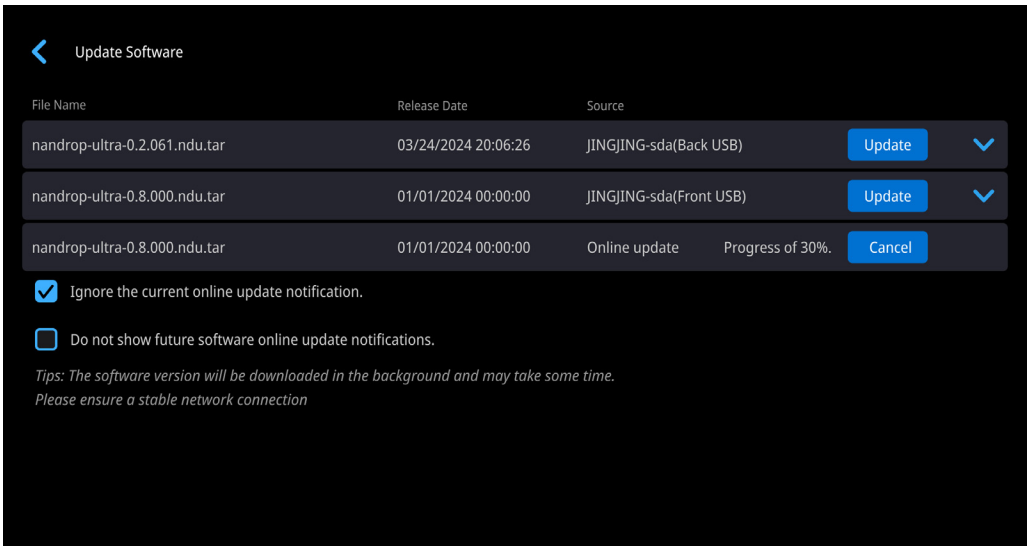
## Update Software

Quickly and easily download and install the latest NanoDrop Ultra software and release notes from our website. Follow the steps to update or upgrade the software on your local instrument and/or install or update the NanoDrop Ultra software on a personal computer (PC). An Internet connection is required to download software.

### To install or update NanoDrop Ultra software on a PC

1. Insert the USB flash drive containing the installer software into an available USB port on your PC, or open the installation folder downloaded from the internet.
2. Launch **Start.exe** and click **Install**. The software installer will run.

### To install or update NanoDrop Ultra software on the instrument




### Through USB:

1. Copy the .zip file with the new software from your computer to a USB storage device. **Do not attempt to unzip the folder**.
2. Insert the USB device into any USB port on the NanoDrop Ultra instrument.
3. From the instrument home screen, tap the Settings icon, then **Software Update.**
4. Select **Update** in the row with the latest version of software.

### Through Online Update

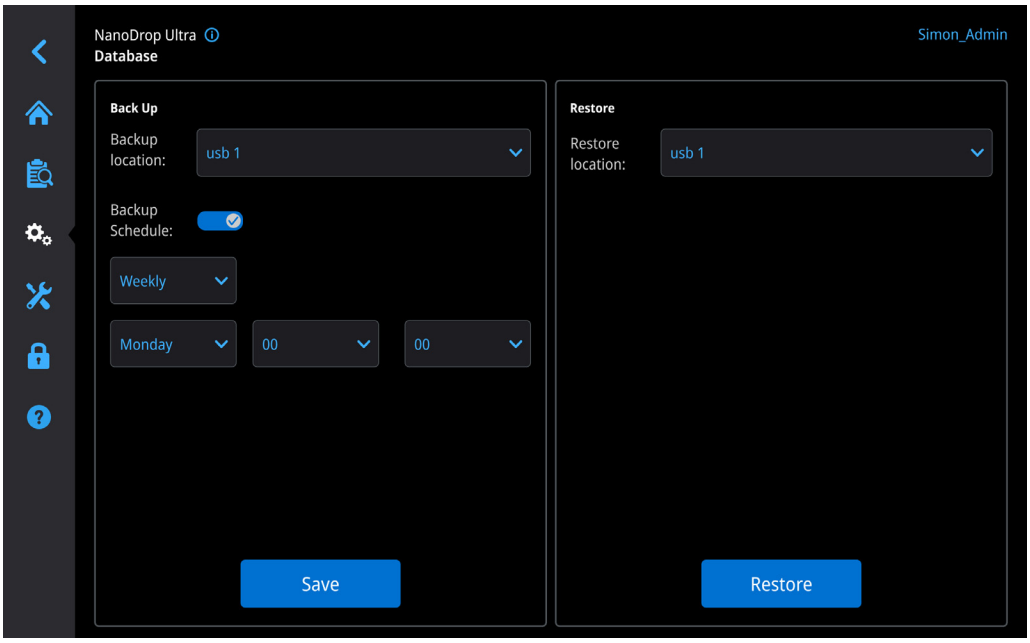
**Note** Instrument must be connected to a network with internet access. When connected to a network with internet access, any time a software update is released, a system alert will be provided to the user to inform them they are able to update.

1. From the instrument home screen, tap the Settings icon > Software Update.

2. Select Update in the row with "Online update" as the source.

## Database Backup and Restore

You can Backup & Restore the internal measurement database to a specified location of your choosing. From the **Settings** screen, select **Database**.




Use the Backup location drop-down menu to select a backup location.

You can configure a database backup as a one-time event, or as a periodic scheduled backup. Specify the date and time of a one-time backup, or toggle **Backup Schedule** to then select the period of the backup.

Select **Save** to save the database to the backup location.

Use the Restore location drop-down menu to select the location of a previously saved Backup file, then select **Restore**.

**Note** Restoring the database will overwrite any data that is not in the Backup file and that data will be lost.

# About Instrument

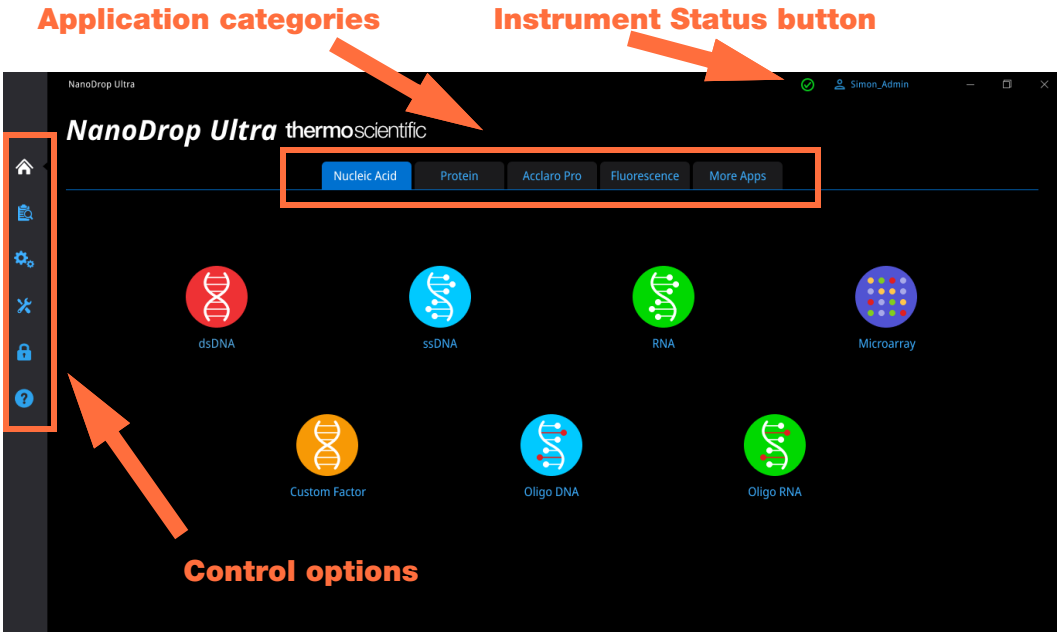
Provides all the same information provided in the System Status information screen with a few exceptions.

| | |
|---|---|
| **Memory Left** | Displays the remaining data storage space on the instrument.<br><br>See “System Status” on page 296 for details on remaining information provided on this screen. |
| **EULA** | Select **EULA** to display Thermo Fisher Scientific's End User License Agreement. Once displayed, the EULA can be exported by selecting **Export**. |

# PC Control Software

Control your NanoDrop Ultra from a PC through a USB A/B connection with the PC control software. You can store or view data acquired with a NanoDrop Ultra instrument on the PC, as well as change instrument settings, and create or edit custom methods. The NanoDrop Ultra local control and PC control software applications are almost identical, a few of the more significant differences will be highlighted below.

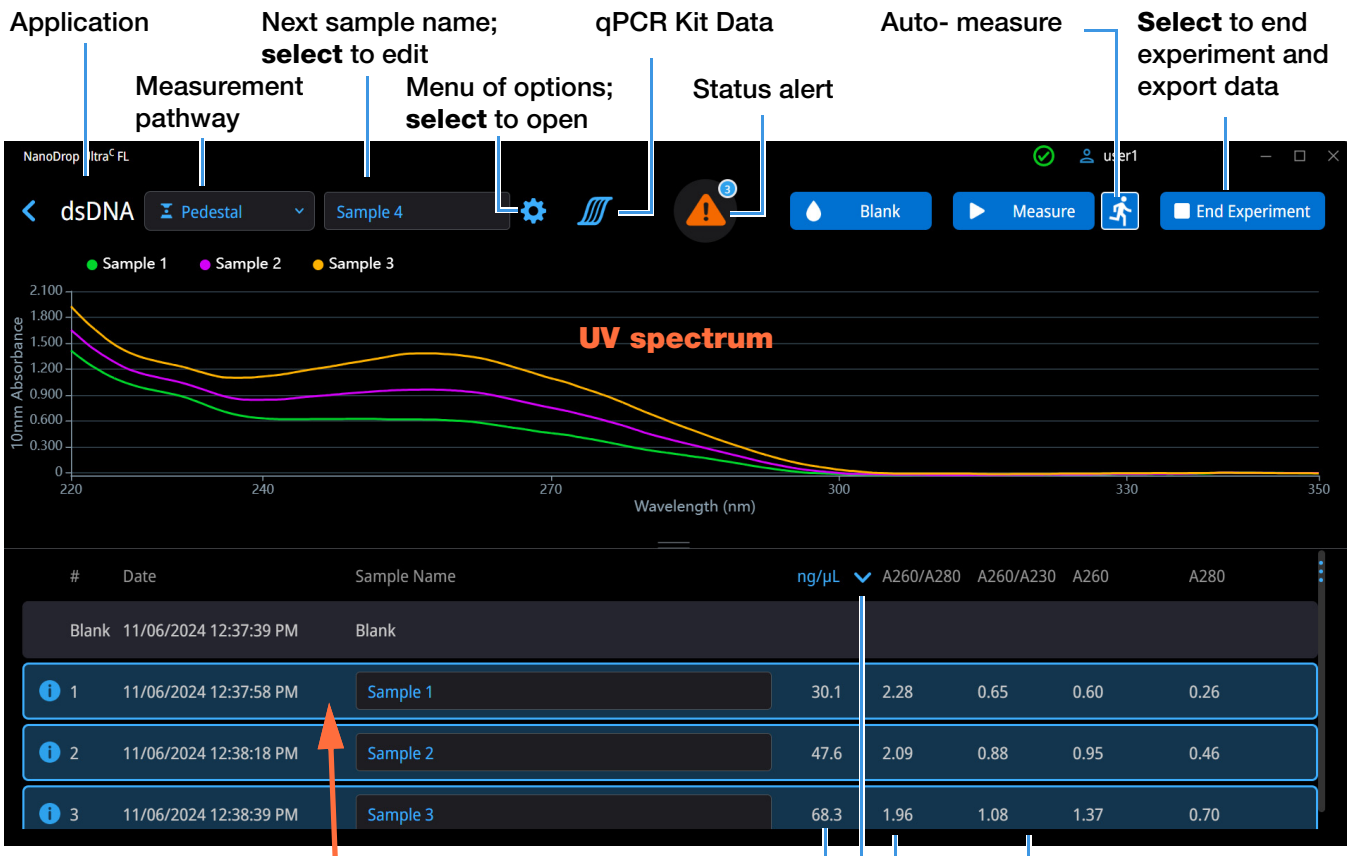
## PC Control Home Screen overview




**PC Control Home Screen**

Select your application from the categorized icons just as you would with the NanoDrop Ultra instrument Home Screen.

## PC Control Measurement Screen




### PC Control Settings

The NanoDrop Ultra PC control settings are very similar to the settings seen on the local instrument. Some settings like Export and About Instrument are not available on the PC control. Additionally, some settings like System Settings have limited options in comparison to the local control software as some of the settings are pulled from the PC.

To access Thermo Fisher Scientific's End User License Agreement, open **System Status** and select **EULA**. Once displayed, the EULA can be exported by selecting **Export**.

10

# Maintenance

# Maintenance Schedule

## Daily Maintenance

- Clean pedestals with deionized water

## Periodic Maintenance

- Clean touchscreen
- Clean and recondition pedestals

## Every 6 Months

- Clean and recondition pedestals
- Run Intensity Check
- Run Performance Verification
- Run Pedestal Image Check
- Run Cuvette Check
- Run Fluorescence Performance Check
- Run Acclaro Pro Check
- Export Logs

If you are experiencing an issue with your system, refer to the troubleshooting information. If the issue persists, contact us. If you are outside the U.S.A. and Canada, please contact your local distributor.

If your instrument requires maintenance or repair, contact us or your local distributor.

# Cleaning the Touchscreen

**Note** To avoid causing permanent damage to the touchscreen, **do not**:

- clean the touchscreen with abrasive material such as paper towel
- apply excessive pressure
- spray liquid directly onto the touchscreen
- apply lubricant to the touchscreen slide mechanism

### To clean the touchscreen

Gently wipe the touchscreen with a soft, lint-free cloth such as microfiber.

If necessary, use a cleaner intended for glass LCD displays and follow the manufacturer's recommendations.

# Maintaining the Pedestals

The pedestals require periodic maintenance to maintain measurement integrity. Time lines and procedures for cleaning and reconditioning the pedestals are provided below.

## Cleaning the Pedestals

To avoid carryover and cross contamination, clean the pedestals before the first blank or sample measurement and at the end of each measurement. Additional cleaning (see below) or reconditioning may be required for periodic maintenance.

**Note**

- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestal as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

**Note** Solutions containing detergent or isopropyl alcohol may uncondition the pedestals. If these are required for sample analyses, follow immediately with 3–5 µL DI $H_2O$.

### Supplies needed

- lint-free laboratory wipes
- deionized water (DI $H_2O$)
- for thorough cleaning: PR-1 kit or 0.5M HCl


### To clean the pedestals between measurements

Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.

### To clean the pedestals between users

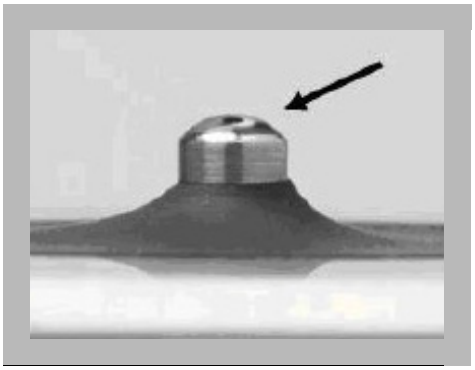
1. Lift the arm and clean both pedestals with a new laboratory wipe.
2. Pipette 3-5 µL DI $H_2O$ onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean both pedestals with a new wipe.

**Tip**: When thorough cleaning is required (for example, to remove dried sample left on the pedestals), clean and recondition the pedestals using PR-1 compound. If you do not have PR-1, you can also substitute 0.5M HCl for the DI $H_2O$ in the procedure above and follow with 3 µL DI $H_2O$.

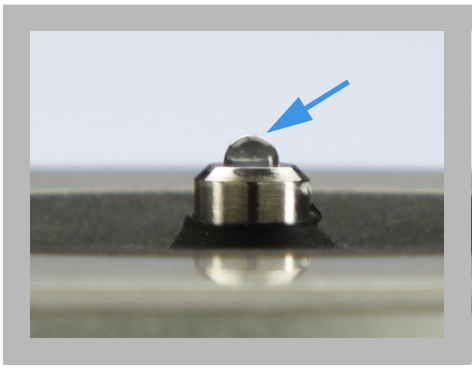
# Reconditioning the Pedestals

The pedestal surfaces may lose their “conditioned” properties over time, especially after measurements with isopropyl alcohol or solutions that contain surfactants or detergents such as the Bradford reagent. An unconditioned pedestal causes droplets on the lower pedestal to “flatten out,” preventing proper formation of the liquid column when the arm is lowered. The resulting spectrum may look “rough” or “jagged.”

If samples flatten out on the pedestal (rather than “beading up” or forming a rounded droplet) or the liquid column breaks during a measurement, recondition the pedestals.



**Unconditioned pedestal**
(droplet flattens out)



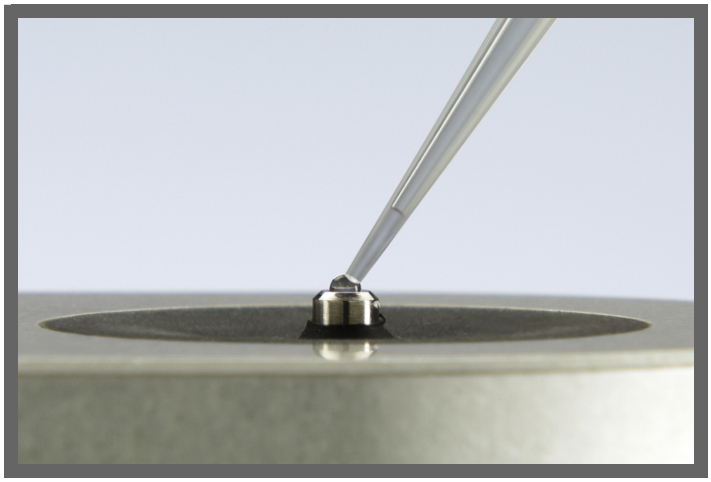
**Properly conditioned pedestal**
(droplet beads up)

## Supplies needed

- lint-free laboratory wipes
- PR-1 pedestal reconditioning kit (available from us or a local distributor)
- calibrated precision pipettor (0-2 µL)
- canned air

## To recondition the pedestals





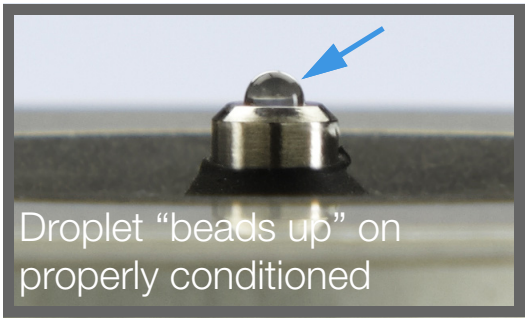
1. Open the container of PR-1 compound and use the provided applicator to remove a pin-head sized amount of the compound.
2. Apply a thin, even layer of reconditioning compound to the surface of the upper and lower pedestal.

   Wait 30 seconds for the PR-1 compound to dry.
3. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal.

   **Notice**: Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.

   **Tip**: Black residue on the wipe is normal.
4. Repeat step 3 with a new folded wipe until all residue is removed and the pedestals buff clean.
5. Use canned air to remove any paper residue from the pedestals.
6. Pipette 1 µL DI $H_2O$ onto the lower pedestal.

   The DI $H_2O$ should “bead up” or form a rounded droplet.



**Tip** The PR-1 pedestal reconditioning compound is the easiest way to recondition the pedestals. If you don't have a PR-1 kit, follow these steps:

1. Lift the instrument arm and pipette 3 µL 0.5M HCl onto the lower pedestal.
2. Lower the arm and wait 2–3 minutes.
3. Lift the arm and clean both pedestals with a new laboratory wipe.
4. Pipette 3 µL DI $H_2O$ onto the lower pedestal.
5. Lower the arm and wait 2–3 minutes.
6. Lift the arm and clean both pedestals with a new wipe.

   **NOTICE:** Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.
7. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal at least 50 times.
8. Use canned air to remove any paper residue from the pedestals.

# Decontaminating the Instrument

Decontaminate the instrument after measurements with samples that contain hazardous materials and before returning the instrument to us for maintenance or repair.

**Note** If your instrument requires maintenance or repair, contact us or your local distributor.

**Note**

- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

### Supplies needed

- lint-free laboratory wipes
- deionized water (DI $H_2O$)
- 0.5% sodium hypochlorite solution (1:10 dilution of commercial bleach, freshly prepared)
- pipettor

#### To decontaminate the pedestals

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Pipette 2–3 µL diluted bleach solution (see Supplies needed) onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean both pedestals with a new wipe.
5. Pipette 3–5 µL DI $H_2O$ onto the lower pedestal.
6. Lower the arm and wait 2–3 minutes.
7. Lift the arm and clean both pedestals with a new wipe.


#### To decontaminate the instrument surfaces

1. Dampen a clean, soft cloth or laboratory wipe with the diluted bleach solution (see Supplies needed) and use it to gently wipe the outside surfaces of the instrument.
2. Use a clean cloth or wipe dampened with DI $H_2O$ to remove the bleach solution.

## Maintaining the Cuvette Sampling System

The cuvette sampling system is included only with the NanoDrop Ultra$^C$ and NanoDrop Ultra$^C$ FL model instruments. For information about compatible cuvettes, see Measuring a Sample using a Cuvette.

**Note** Clean and dry cuvettes after each measurement. Use cuvettes that are free of scratches and avoid fingerprints which may affect results.

**Note** Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.

### To maintain the cuvette sampling system

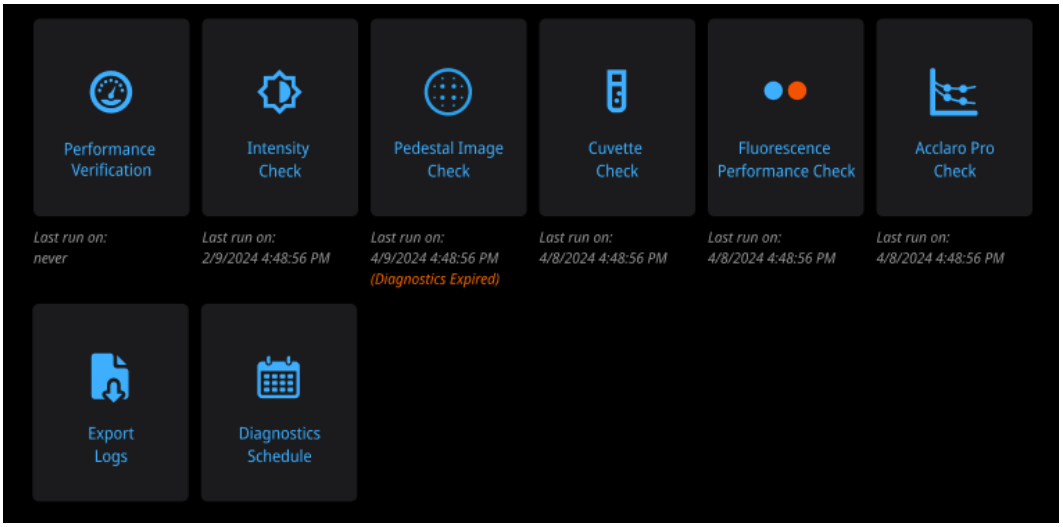
- Keep the instrument arm closed when the instrument is not in use.
- Use canned air to remove any dust from the cuvette holder.
- Clean up any spills inside the cuvette holder with a new laboratory wipe.

To clean and maintain cuvettes, follow the recommendations of the cuvette manufacturer.

# Instrument Diagnostics

Every 6 months, run the following performance and quality checks to verify instrument operation.

Diagnostics can be performed using the NanoDrop Ultra local instrument software or the PC control software. **Performance Verification**, **Intensity Check**, **Pedestal Image Check**, **Cuvette Check**, **Fluorescence Performance Check**, and **Acclaro Pro Check** are all accessible from **Diagnostics** on the software home screen.




**Diagnostic options**

| | |
|---|---|
| Performance Verification: | Run a pedestal performance verification test using PV-1 |
| Intensity Check: | Run an intensity check for the cuvette or pedestal |
| Pedestal Image Check: | Run a pedestal image check |
| Cuvette Check: | Run a cuvette transmittance test |
| Fluorescence Performance Check: | Run a fluorescence performance test using FL-1 |
| Acclaro Pro Check: | Run an Acclaro Pro performance check using PV-1 |
| Export Logs: | Export instrument related log files for troubleshooting |
| Diagnostics Schedule | Establish a diagnostic schedule that provides timely alerts, reminding you when the next diagnostic checks are due |

## Intensity Check

It is recommended to run an Intensity Check every 6 months to verify operation of the instrument's internal components. The test measures the intensity of light from the xenon source through the instrument to verify that throughput, wavelength accuracy, and bias are within specifications. The test is automatically performed using the pedestal and the cuvette optical paths.

### Supplies needed

- lint-free laboratory wipes

### To run Intensity Check

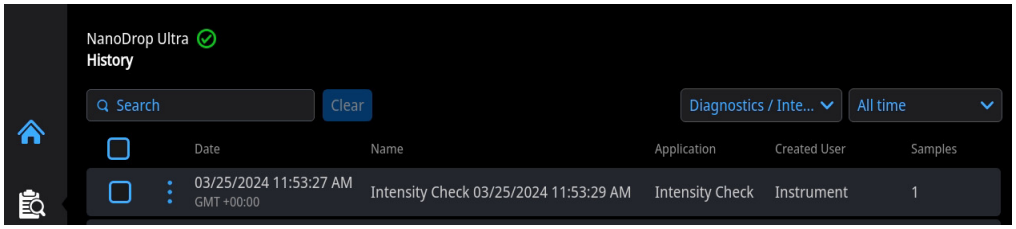
1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Remove any cuvette from the cuvette holder.
3. Lower the arm.
4. From the home screen, select the **Diagnostics** icon, followed by **Intensity Check**.
5. When using the NanoDrop Ultra$^{C}$ or NanoDrop Ultra$^{C}$ FL, select which pathway to test.
   - Use the dropdown menu at the top of the screen to select **Pedestal** or **Cuvette**.
6. Tap or select **Measure** and wait for the measurement to complete.

   Here is an example of a typical Intensity Check result screen from the NanoDrop Ultra local control software.


**Swipe screen left to view detailed results**

7. To rerun the Intensity Check, select **Measure**.
8. When finished, select **End Experiment**.

After the test is completed, the results are available from the History (see example below). See Manage identifiers on the instrument for details.




### To interpret Intensity Check results

If one of these indicators:

- UV
- Visible
- Bias

has an adjacent orange exclamation mark instead of the green check marks shown above, clean the pedestals with deionized water and then repeat the Intensity Check. When using the PC control software, the status column in the results table will display "Pass" or "Fail" for each criterion and the column beside it will display any applicable details.

If an orange exclamation mark appears next to the Bias indicator, or it's status is marked as fail, make sure the room is within the temperature specifications for the instrument.

If the Intensity Check fails again, contact us.

## Performance Verification

It is recommended to run a Performance Verification every 6 months to confirm pathlength accuracy is within specifications.

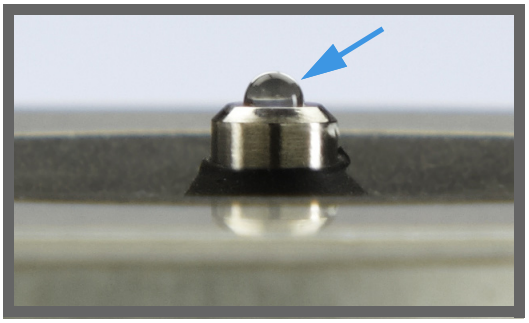
### Supplies needed

- lint-free laboratory wipes
- deionized water (DI $H_2O$)
- calibrated precision pipettor (0–2 µL)
- PV-1 performance verification solution (liquid photometric standard available only from us or a local distributor)
- laboratory gloves

**Note** The PV-1 solution comes in a single-use ampoule. Before you open the ampoule, ensure that the solution is thoroughly mixed by vigorously shaking/inverting the ampoule. Allow the solution to collect in the bottom portion of the ampoule. If needed, gently tap or flick the ampoule. After the ampoule is opened, its contents must be used within one hour. Pipette directly from the ampoule; do not transfer the solution.

### Before you begin

First make sure the pedestals are properly conditioned. To test pedestal conditioning, clean the pedestals with a new laboratory wipe, then pipette 1 µL DI $H_2O$ onto the lower pedestal. The droplet should "bead up" as shown below. If it does not, recondition both pedestals.

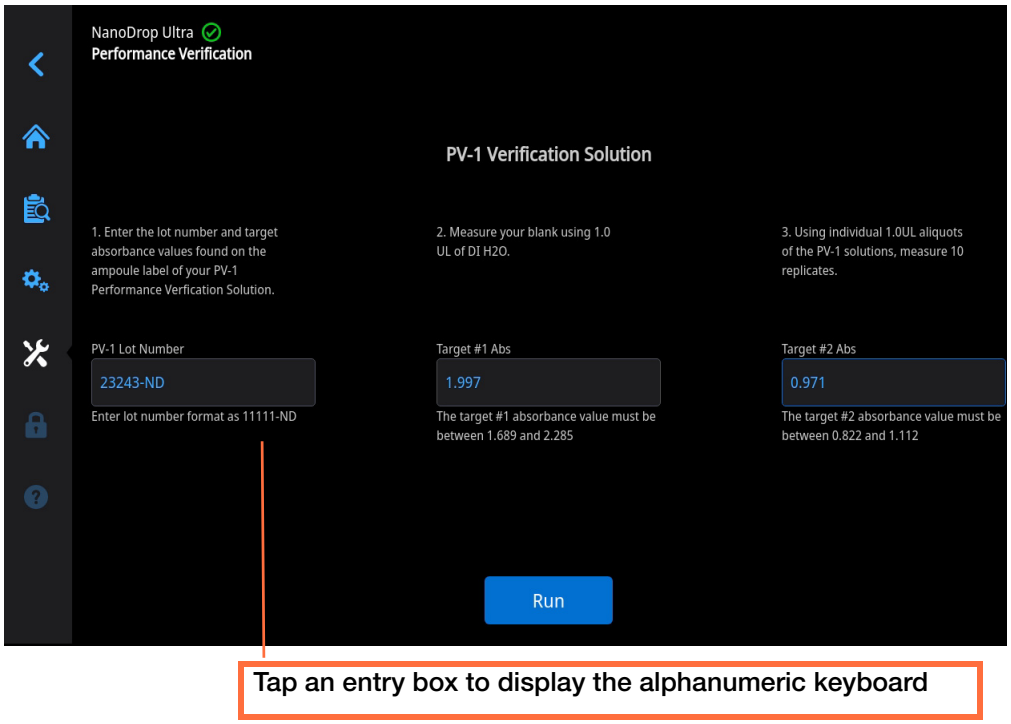


**Droplet "beads up" on properly conditioned pedestal**

### To run the performance verification

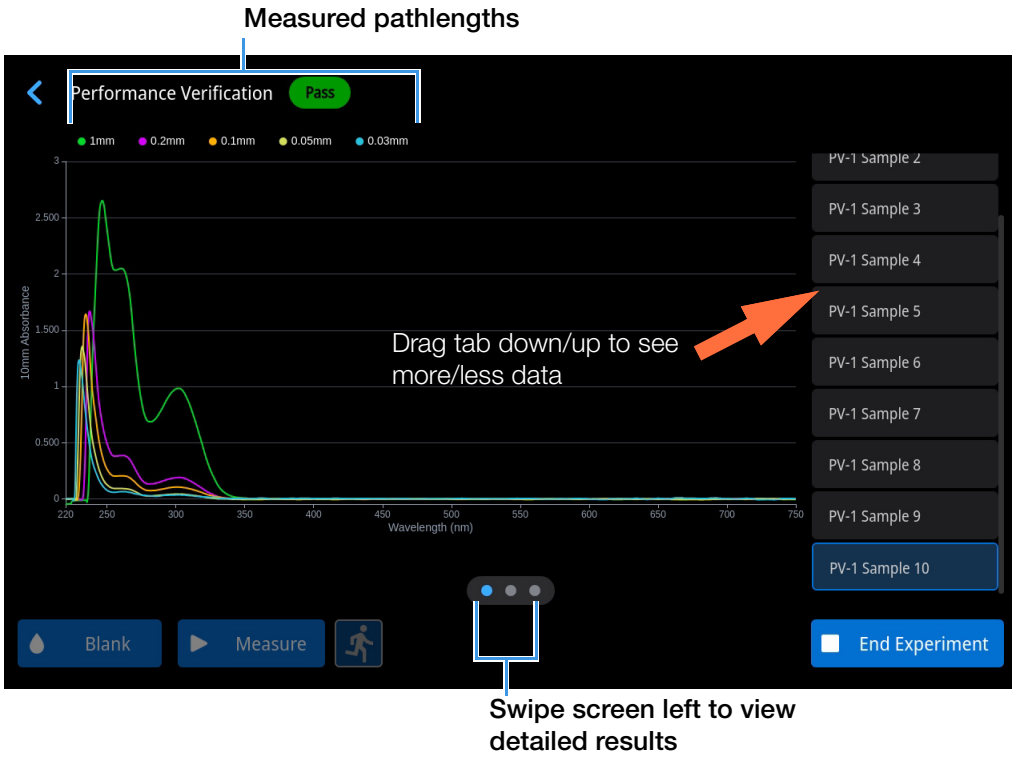
1. From the home screen, select the Diagnostics icon ,followed by **Performance Verification**.

   A message asks for the PV-1 lot number and target absorbance values.



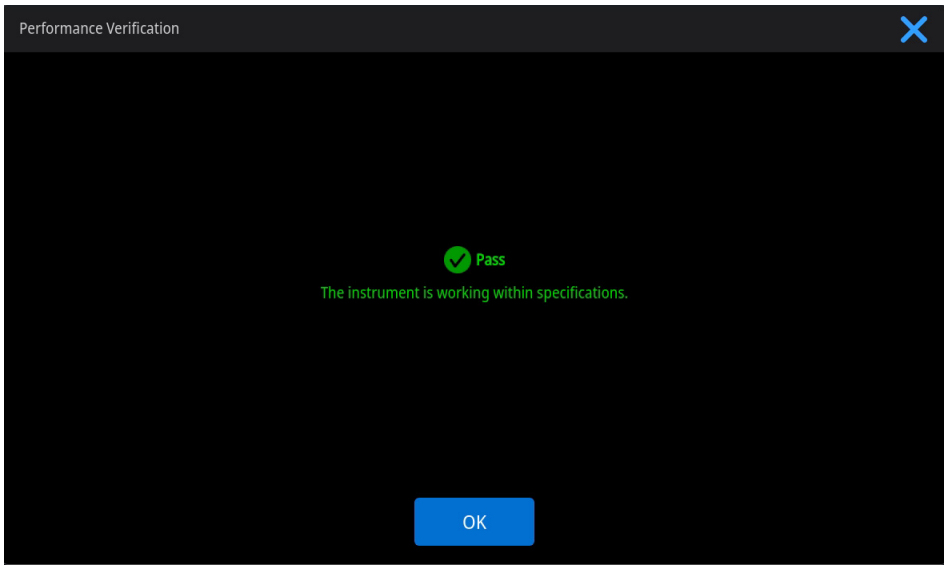

2. Enter the lot number of the PV-1 ampoule (found on the PV-1 ampoule label) that is going to be used for this test. Select **Done** when using the local instrument control to close the keyboard.
3. Enter each lot-specific target absorbance value from the label on the PV-1 ampoule in its associated entry box and then select **Done**.
4. Once all values have been entered, select **Run**.
5. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
6. Pipette 1 µL DI $H_2O$ onto the lower pedestal, lower the arm and select **Blank**.
7. Lift the arm and clean both pedestals with a new wipe.
8. Ensure PV-1 solution is thoroughly mixed by vigorously shaking/ inverting the ampoule. Allow the solution to collect in the bottom portion of the ampoule. If needed, tap or flick the ampoule.
9. Carefully snap off the top portion of the ampoule using a plastic ampoule cracker. Discard top along with ampoule cracker (use proper safety precautions for disposal).
10. Pipette 1 µL PV-1 solution onto the lower pedestal and start the sample measurement:
    - If Auto-Measure is On, lower arm
    - If Auto-Measure is Off, lower arm and select **Measure**.

After the measurement, the software displays the results. Here is an example of the performance verification result screen.

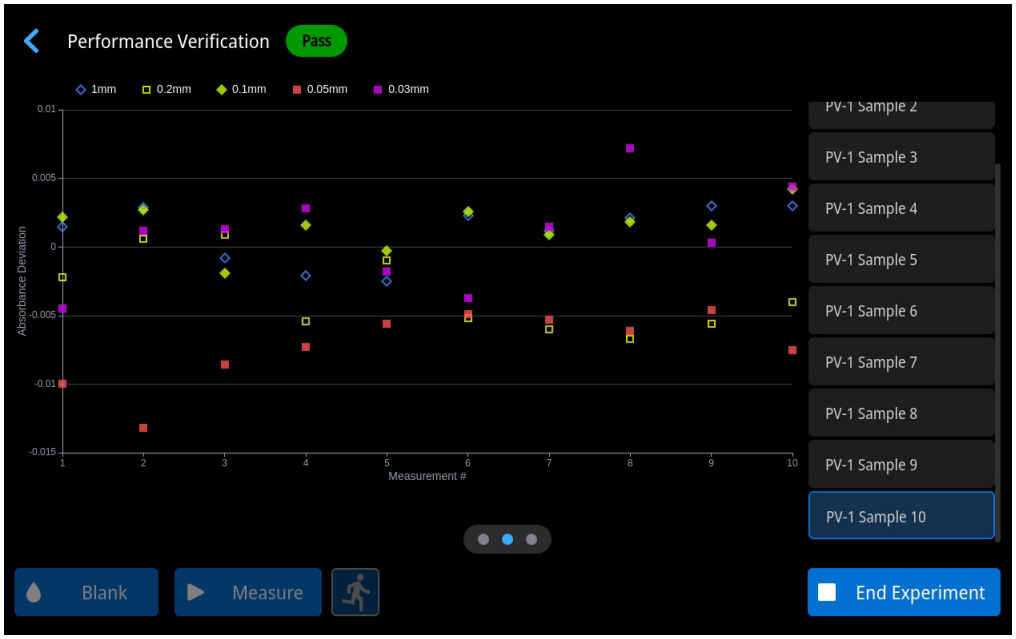



11. Repeat step 10 to measure the PV-1 solution nine more times using a new 1 µL aliquot for each measurement and cleaning both pedestals after each measurement. After each measurement, a new sample result is added to the display.

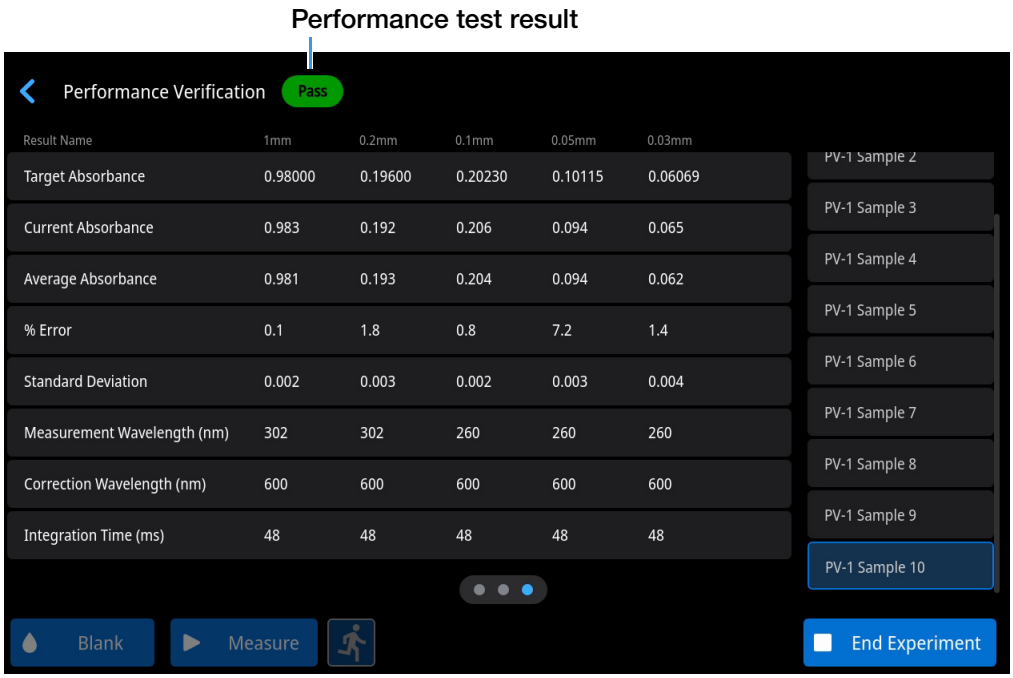
    After the tenth measurement, a message containing the overall result of the performance verification will be displayed. Below is an example of what would be seen after passing a test. Select **OK** to see a more detailed summary of the results.




Swipe the screen left to see a summary of the 10 sample results.

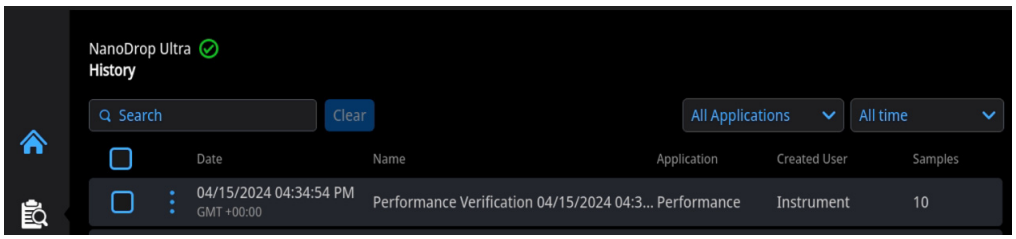


Swipe left again to see additional measurement details, along with the overall test result.




12. When finished, select **End Experiment** and clean the pedestals with 3–5 µL DI $H_2O$.

    After the test is completed, the results are available from the History (see example below). See Manage identifiers on the instrument for details.




## To interpret performance verification results

The overall result of the test will be displayed at the top of the screen as a Green "Pass"/ Orange "Conditional Pass"/ Red "Fail".

- If results are not within specifications, repeat procedure using 2 µL aliquots of PV-1.
- If results fail to meet specifications using 2 µL aliquots, contact us.
- A conditional pass means that the instrument is working slightly outside of our factory specifications, but not yet failing. With a conditional pass, it is left up to the end user to determine if those results are acceptable for their intended purposes.

# Pedestal Image Check

Run the Pedestal Image Check periodically to verify the instrument's column sensor which monitors for possible errors such as an empty column or bubbles in a sample. The Pedestal Image Check can be used for routine quality control purposes. It also provides important diagnostic information if a detection system component fails.

### Supplies needed

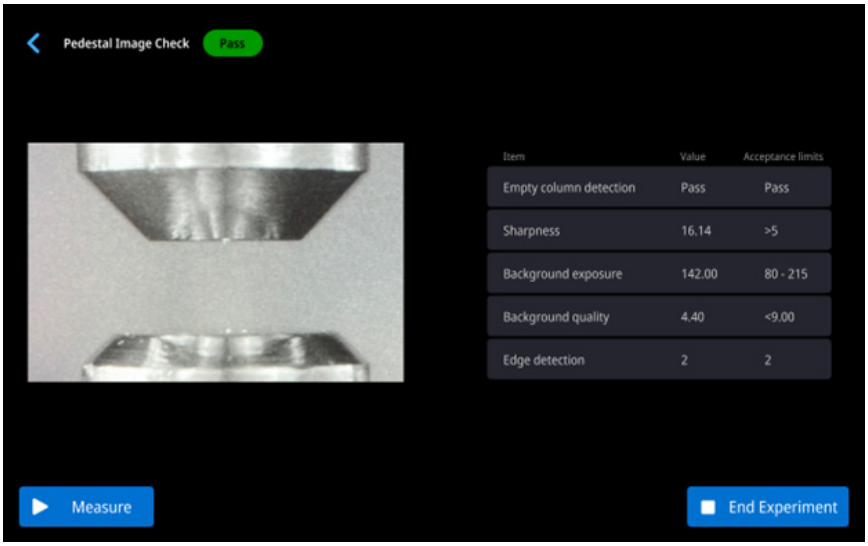
- lint-free laboratory wipes

### To run the Pedestal Image Check

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Lower the arm.
3. From the instrument home screen, select  , (Diagnostics) and then select **Pedestal Image Check**.
4. Select **Measure**.

   The instrument runs a series of tests to check pedestal position and image quality. After the measurements are completed, the results are displayed. The display of a Pass result at the top of the screen indicates the instrument passed the Pedestal Image Check.
5. When finished, select **End Experiment**.

### To interpret Pedestal Image Check results




If the Pedestal Image Check displays a Fail result, follow the on-screen instructions to fix any possible problems. Then rerun the Pedestal Image Check. If the instrument fails again, contact us.

# Cuvette Check

Run the Cuvette Check test every 6 months to confirm photometric accuracy is within specification when using the cuvette mode. This module is only available on the NanoDrop Ultra$^{C}$ and NanoDrop Ultra$^{C}$ FL instruments.

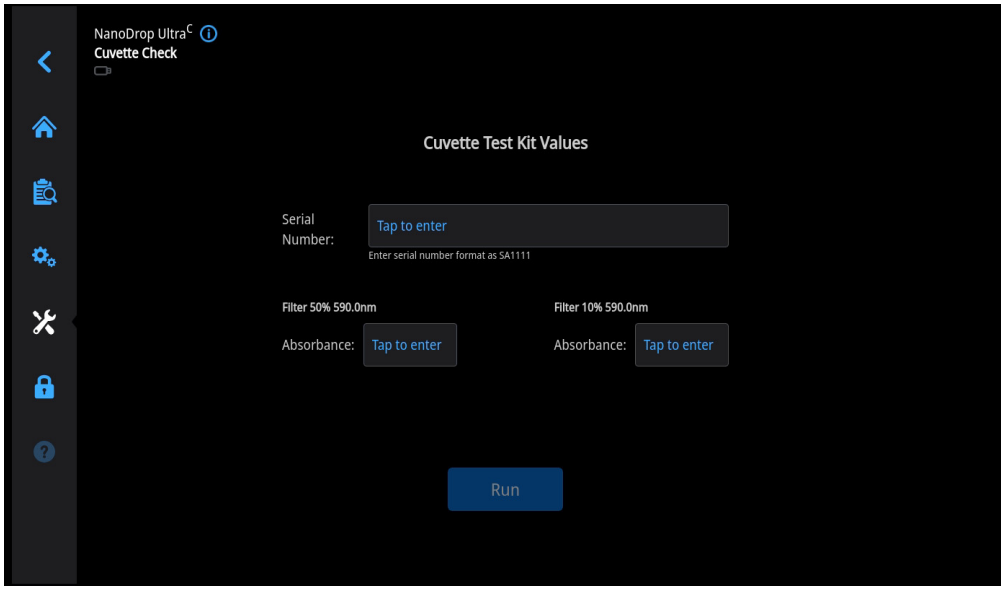
### Supplies needed

- Cuvette Qualification Filter Kit for NanoDrop Instruments (PN 840-257300)
- NIST-Traceable Certified 10% Transmittance (at 590 nm) Neutral Density Filter
- NIST-Traceable Certified 50% Transmittance (at 590 nm) Neutral Density Filter

## To run the Cuvette Check

1. From the home screen, select  , (Diagnostics) and then select **Cuvette Check**.

   A message asks for the serial number of the cuvette qualification filter kit and the target absorbance values of each filter at 590 nm.



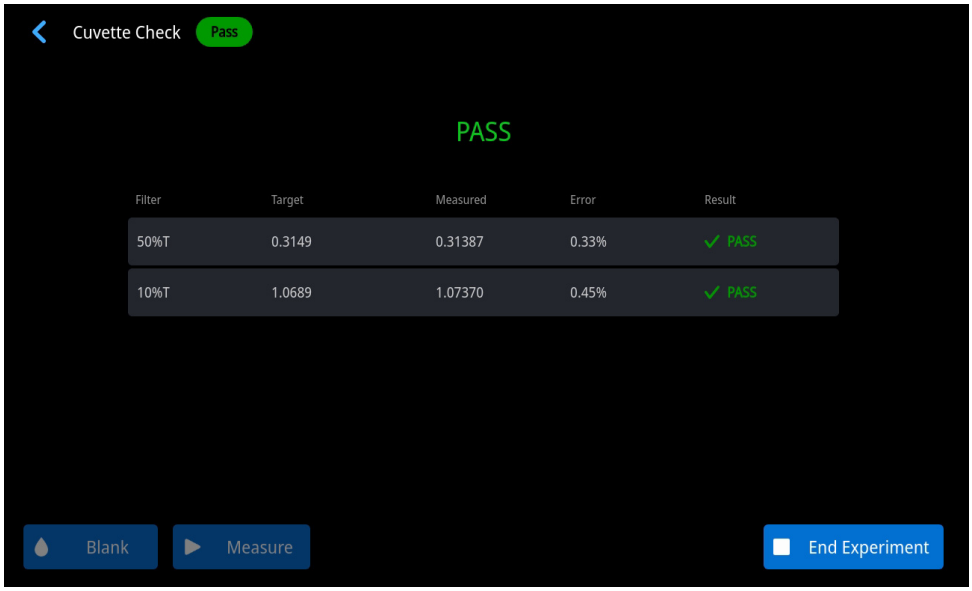

2. Enter the serial number of the qualification filter kit and the lot-specific target absorbance at 590 nm for each filter into their respective box. When using the local instrument software, select **Done** to close the number pad window for each entry.

   This information can be found on the certificate of calibration provided with the cuvette qualification filter kit.

3. After all values have been input, select **Run**.

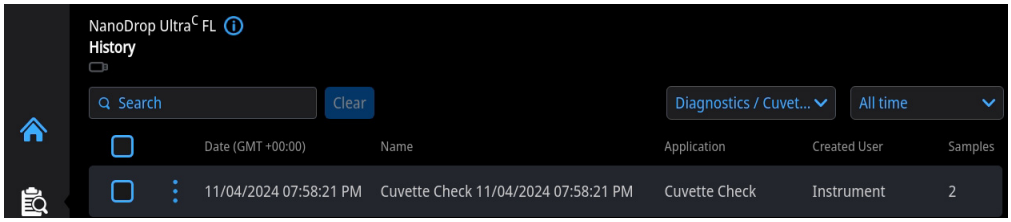
4. Ensure the cuvette holder is empty and clear from obstruction.
5. With no cuvette inside the cuvette holder, select **Blank** to record the blank measurement. A blank filter is not needed.
6. Load the 50%T filter into the cuvette holder ensuring that the filter is oriented correctly, select **Measure**.
   – The arrow etched into the assembly indicates the direction of the light path.
7. Load the 10%T filter into the cuvette holder ensuring that the filter is oriented correctly, select **Measure**.
8. After each measurement, the software will display the result for the measured filter in the results table with either a green checkmark Pass or a red X Fail. If both filters provide passing results, an overall Pass result will be displayed.

   Here is an example of the **Cuvette Check** result screen from the NanoDrop Ultra local control software.




9. If one or both filters fail, the cuvette check will also fail. In the case of a failed cuvette check, immediately repeat the test.
10. When finished, select **End Experiment**. Remove any remaining filters from the cuvette holder.

    After the test is completed, the results are available from the **History** (see example below). See **Manage identifiers** on the instrument for details.



### To interpret Cuvette Check results

If your instrument failed the cuvette check more than once, contact us.

## Fluorescence Performance Check

Run the Fluorescence Performance Check every 6 months to confirm fluorescence performance and accuracy are within specifications. This module is only available on the NanoDrop Ultra FL and NanoDrop Ultra$^{C}$ FL instruments.

Supplies needed

- FL-1 Fluorescence Verification Assay Kit (CHEM-FL1-KIT)
  - Blank reagent solution
  - Green fluorescence reagent
  - Far-red fluorescence reagent
- plastic tubes, 0.2 mL, flat cap or similar
- lint-free laboratory wipes
- deionized water (DI H2O)
- calibrated precision pipettor (0-2 µL)
- laboratory gloves
- vortex mixer
- mini centrifuge

#### Before you begin

Set up three sterile plastic amber tubes for each of the three reagents provided in the FL-1 Fluorescence Verification Kit (Blank reagent, Green Fluorescence Reagent, and Far Red Fluorescence Reagent) and label them Blank, Green Standard 2, and Far Red Standard 2.

Set up two additional sterile plastic amber tubes for dilutions of both Green Fluorescence Reagent and Far Red Fluorescence Reagent and label them Green Standard 1 and Far Red Standard 1.

Blank Tube

- Add 50 µL of Blank Reagent to the Blank tube.

Green Standard 2 Tube

- Add 22 µL of Green Fluorescence Reagent.

Far Red Standard 2 Tube

- Add 30 µL of Far Red Fluorescence Reagent.

Green Standard 1 Tube

- Transfer 18 µL of Blank Reagent from the tube labeled Blank.
- Transfer 2 µL of Green Standard 2 and vortex for 3-5 seconds.
- Centrifuge briefly, a 10-fold diluted Green Fluorescence Reagent is ready.

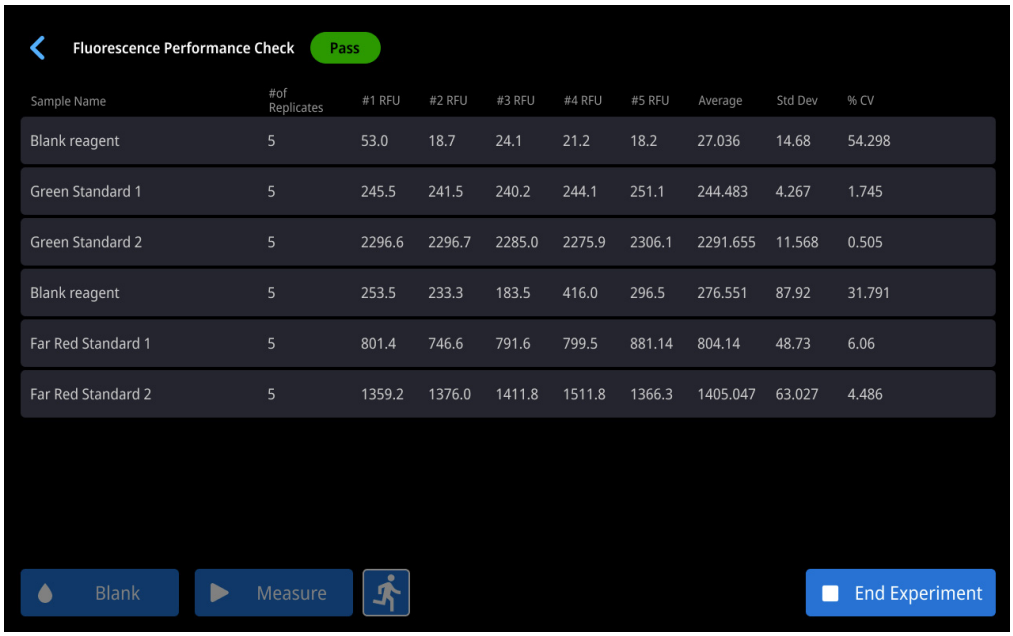
Far Red Standard 1 Tube

- Transfer 10 µL of Blank Reagent from the tube labeled Blank.
- Transfer 10 µL of Far Red Standard 2 and vortex for 3-5 seconds.
- Centrifuge briefly, a 2-fold diluted Far-red Fluorescence Reagent is ready.

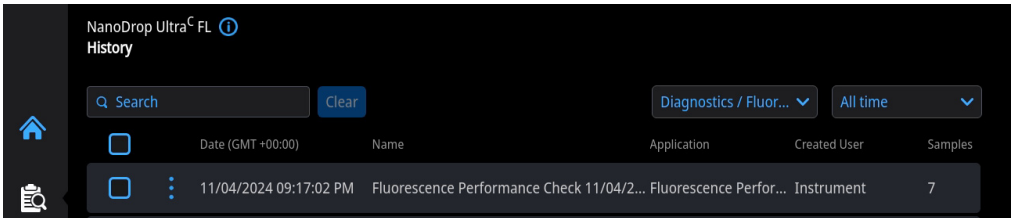
## To run the Fluorescence Performance Check

1. From the home screen, select the Diagnostics icon  , followed by **Fluorescence Performance Check**.
2. A message will appear stating the test requires the use of the FL-1 Fluorescence Verification Kit, select **Run**.
3. Ensure nothing is on the pedestal and select **Blank**.
4. Pipette 2 µL of the Blank Reagent from the Blank tube onto the lower pedestal.
   a. If Auto-Measure is ON  , lower arm.
   b. If Auto-Measure is OFF  , lower arm and select **Measure**.
5. Lift the arm and clean both pedestals with a new wipe.
6. Repeat steps 4 & 5 for a total of five measurements.
7. Pipette 2 µL of the diluted Green Fluorescence Reagent from the Green Standard 1 tube onto the lower pedestal.
   a. If Auto-Measure is ON, lower arm.
   b. If Auto-Measure is OFF, lower arm and select **Measure**.
8. Lift the arm and clean both pedestals with a new wipe.
9. Repeat steps 7 & 8 for a total of five measurements.
10. Pipette 2 µL of the Green Fluorescence Reagent from the Green Standard 2 tube onto the lower pedestal.
    a. If Auto-Measure is ON, lower arm.
    b. If Auto-Measure is OFF, lower arm and select **Measure**.

11. Lift the arm and clean both pedestals with a new wipe.
12. Repeat steps 10 & 11 for a total of five measurements.
13. Pipette 2 µL of the Blank Reagent from the Blank tube onto the lower pedestal.
    a. If Auto-Measure is ON, lower arm.
    b. If Auto-Measure is OFF, lower arm and select **Measure**.
14. Lift the arm and clean both pedestals with a new wipe.
15. Repeat steps 13 & 14 for a total of five measurements.
16. Pipette 2 µL of the diluted Far-Red Fluorescence Reagent from the Far Red Standard 1 tube onto the lower pedestal.
    a. If Auto-Measure is ON, lower arm.
    b. If Auto-Measure is OFF, lower arm and select **Measure**.
17. Lift the arm and clean both pedestals with a new wipe.
18. Repeat steps 16 & 17 for a total of five measurements.
19. Pipette 2 µL of the Far-Red Fluorescence Reagent from the Far Red Standard 2 tube onto the lower pedestal.
    a. If Auto-Measure is ON, lower arm.
    b. If Auto-Measure is OFF, lower arm and select **Measure**.
20. Lift the arm and clean both pedestals with a new wipe. Repeat steps 19 & 20 for a total of five measurements.




| Sample Name | #of Replicates | #1 RFU | #2 RFU | #3 RFU | #4 RFU | #5 RFU | Average | Std Dev | % CV |
|---|---|---|---|---|---|---|---|---|---|
| Blank reagent | 5 | 53.0 | 18.7 | 24.1 | 21.2 | 18.2 | 27.036 | 14.68 | 54.298 |
| Green Standard 1 | 5 | 245.5 | 241.5 | 240.2 | 244.1 | 251.1 | 244.483 | 4.267 | 1.745 |
| Green Standard 2 | 5 | 2296.6 | 2296.7 | 2285.0 | 2275.9 | 2306.1 | 2291.655 | 11.568 | 0.505 |
| Blank reagent | 5 | 253.5 | 233.3 | 183.5 | 416.0 | 296.5 | 276.551 | 87.92 | 31.791 |
| Far Red Standard 1 | 5 | 801.4 | 746.6 | 791.6 | 799.5 | 881.14 | 804.14 | 48.73 | 6.06 |
| Far Red Standard 2 | 5 | 1359.2 | 1376.0 | 1411.8 | 1511.8 | 1366.3 | 1405.047 | 63.027 | 4.486 |




### To interpret Fluorescence Performance Check results

The overall result of the test will be displayed at the top of the screen as a Green "Pass" or Red "Fail".

If results are not within specifications, repeat procedure with new dilutions of the FL-1 reagents.

If results fail to meet specifications after the second attempt, contact us.

## Acclaro Pro Check

It is recommended to run the Acclaro Pro Check every 6 months to confirm pathlength accuracy is within specifications for the Acclaro Pro applications.

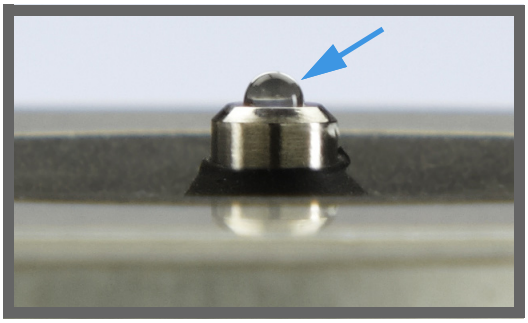
### Supplies needed

- lint-free laboratory wipes
- deionized water (DI H2O)
- calibrated precision pipettor (0-2 µL)
- PV-1 performance verification solution (liquid photometric standard available only from us or a local distributor)
- laboratory gloves

**Note** The PV-1 solution comes in a single-use ampoule. Before you open the ampoule, ensure that the solution is thoroughly mixed by vigorously shaking/inverting the ampoule. Allow the solution to collect in the bottom portion of the ampoule. If needed, gently tap or flick the ampoule. After the ampoule is opened, its contents must be used within one hour. Pipette directly from the ampoule; do not transfer the solution.

### Before you begin

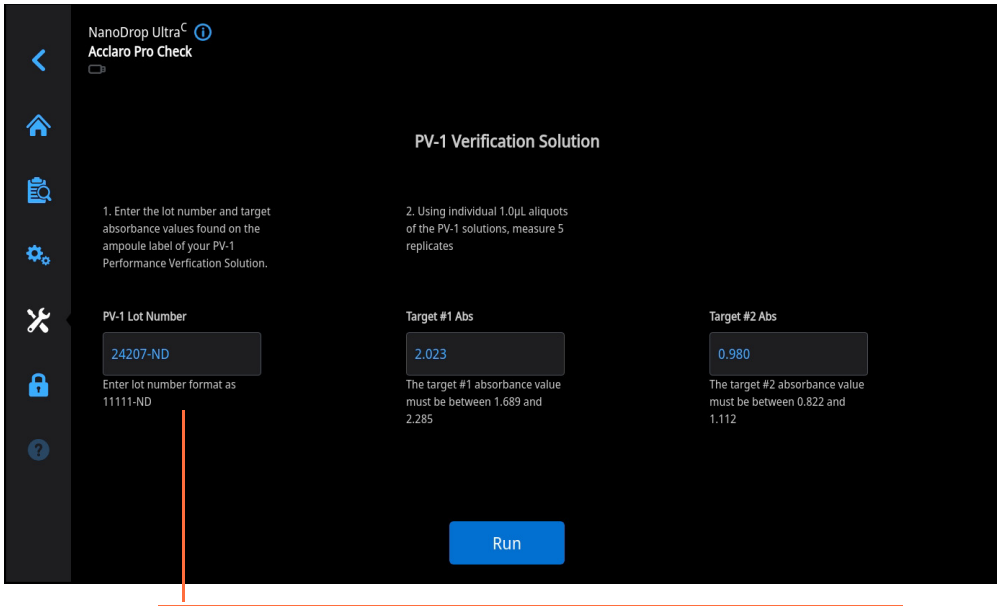
First make sure the pedestals are properly conditioned. To test pedestal conditioning, clean the pedestals with a new laboratory wipe, then pipette 1 µL DI $H_2O$ onto the lower pedestal. The droplet should "bead up" as shown below. If it does not, recondition both pedestals.



**Droplet "beads up" on properly conditioned pedestal**

### To run the Acclaro Pro Check

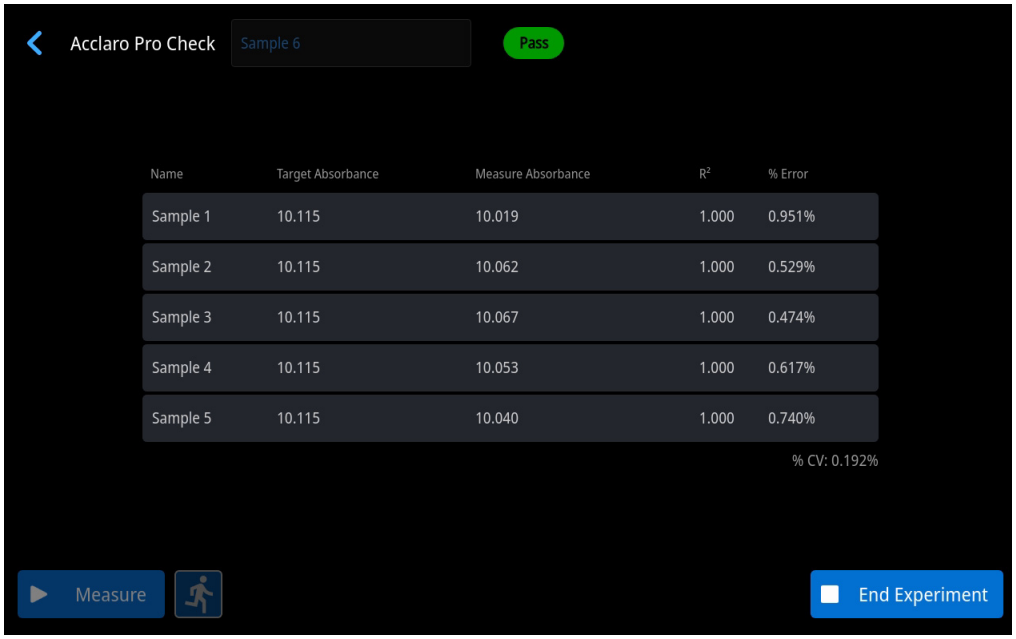
1. From the home screen, select the Diagnostics icon ,followed by **Acclaro Pro Check**. A message asks for the PV-1 lot number and target absorbance values.




2. Enter the lot number of the PV-1 ampoule (found on the PV-1 ampoule label) that is going to be used for this test. Select **Done** when using the local instrument control to close the keyboard.
3. Enter each lot-specific target absorbance value from the label on the PV-1 ampoule in its associated entry box. Select **Done** when using the local instrument control to close the keyboard.

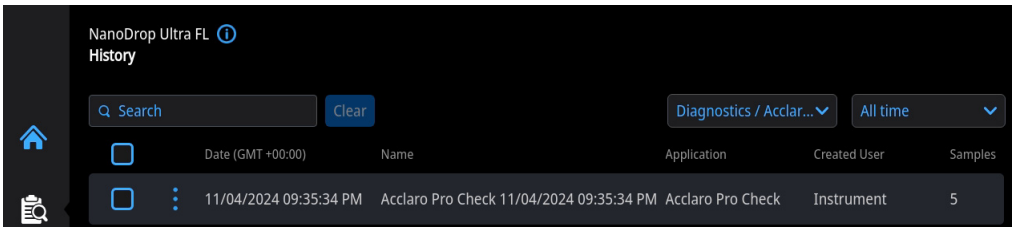
4. Once all values have been entered, select **Run**.
5. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
6. Ensure PV-1 solution is thoroughly mixed by vigorously shaking/ inverting the ampoule. Allow the solution to collect in the bottom portion of the ampoule. If needed, tap or flick the ampoule.
7. Carefully snap off the top portion of the ampoule using a plastic ampoule cracker. Discard top along with ampoule cracker (use proper safety precautions for disposal).
8. Pipette 1 µL PV-1 solution onto the lower pedestal and start the sample measurement:
   - If Auto-Measure is On, lower arm
   - If Auto-Measure is Off, lower arm and select **Measure**.
9. Repeat step 8 to measure the PV-1 solution four more times using a new 1 µL aliquot for each measurement and cleaning both pedestals after each measurement.

   After the fifth measurement, the software displays the results. Here is an example of the Acclaro Pro Check result screen from the NanoDrop Ultra local control software.




10. When finished, select End Experiment and clean the pedestals with 3-5 µL DI H2O.

After the test is completed, the results are available from the **History** (see example below). See **Manage identifiers** on the instrument for details.




### To interpret Acclaro Pro Check results

1. The overall result of the test will be displayed at the top of the screen as a Green "Pass" or a Red "Fail".
   - If results are not within specifications, repeat procedure using 2 µL aliquots of PV-1.
   - If results fail to meet specifications using 2 µL aliquots, contact us.

## Export Logs

The Export Logs feature is a functionality that allows users to retrieve logs generated by the instrument software for diagnostic purposes.

### To export instrument log file

1. From the home screen, select , (diagnostics) and then select **Export Logs**.
2. Select preferred export method and then select **Export**.

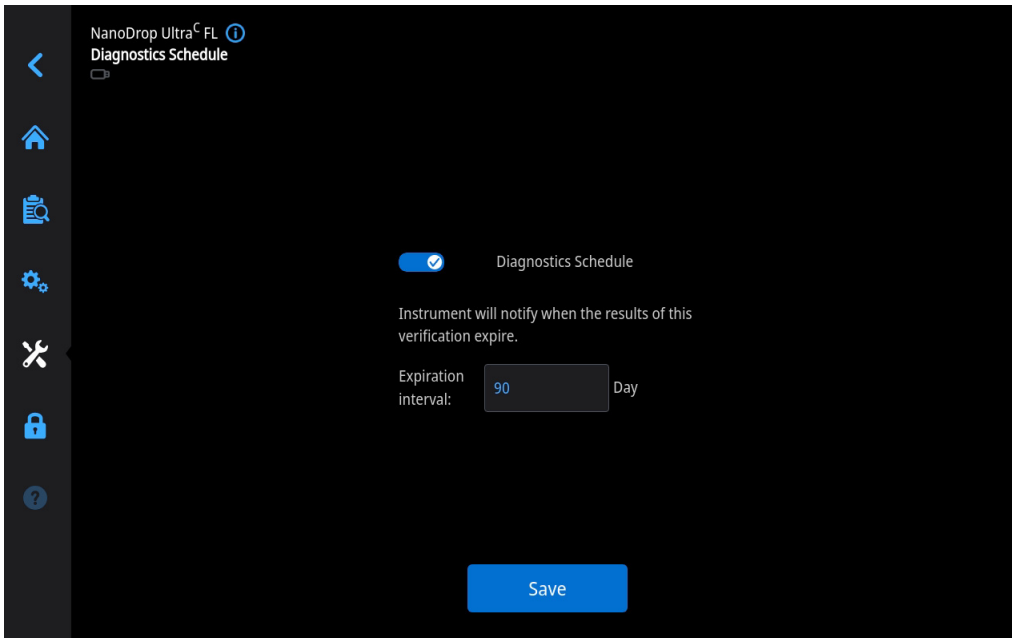
## Diagnostics Schedule

The Diagnostics Schedule feature enables users to set specific intervals for receiving alerts, ensuring timely execution of diagnostic checks on the instrument. The NanoDrop Ultra Spectrophotometers and Fluorometers track the last run date and time for all available diagnostics, providing an automated, reliable system to maintain optimal instrument performance.

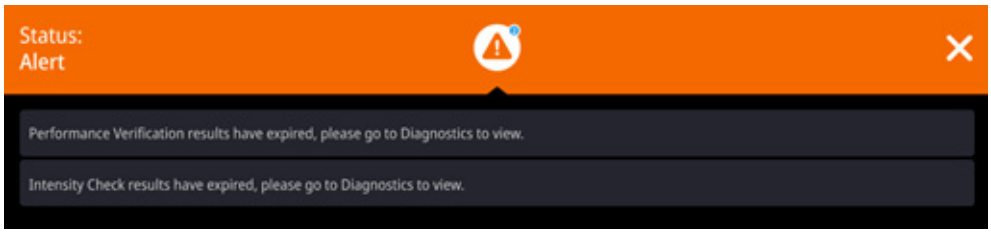
### To enable the diagnostics schedule

1. From the home screen, select , (diagnostics) and then select Diagnostics Schedule.
2. Use the toggle switch next to Diagnostics Schedule to enable or disable the feature.

3. Once enabled, use the entry box besides Expiration interval to define the number of days (90-999) that will pass before a diagnostic test is deemed expired.




Once a diagnostic test has expired, an alert will then trigger to alert message to the user. An example of this alert has been provided below.



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# Safety and Operating Precautions

**Contents**



**Note** Be sure that all persons operating this system read the safety manual first.

# Operating Precautions

**CAUTION** Do not remove the instrument cover. Removing the cover exposes the operator to sharp edges and delicate fiber optic cables. The instrument warranty is void if the cover has been removed.

NanoDrop Ultra spectrophotometers and fluorometers are designed to operate indoors in an environment that meets our specifications. For details, see the site preparation guide for your instrument.

Follow these precautions to avoid damaging your NanoDrop spectrophotometer and fluorometer during use:

- Use a grounded power cord appropriate for your electrical service. If the supplied power cord is incompatible or if it becomes damaged, contact us.
- Do not remove the instrument cover.
- The LCD display uses heat treated, chemical tempered glass and is rugged and difficult to break. However, should the display become cracked or broken, contact us for replacement.
- Use solvents that are compatible with the instrument (see Hazardous Materials)
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestal as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

# Safety Information

Before operating a NanoDrop Ultra instrument, please read the safety information and follow its recommendations for the system.

## Safety and Special Notices

In many cases, safety information is displayed on the instrument itself. The symbol indicates that there is additional safety information in the documentation and failure to heed the safety precautions could result in injury.

**WARNING** Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

**CAUTION** Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

**Note** Follow instructions with this label to avoid damaging the system hardware or losing data.

**Note** Contains helpful supplementary information.

The following table lists some of the safety symbols and their indications that may appear in the user documentation.

| Symbols | Indication |
|---|---|
|  | This is a mandatory action symbol. It is used to indicate that an action shall be taken to avoid a hazard. |
|  | This is a prohibition symbol. The graphic in this symbol is used to alert the user to actions that shall not be taken or shall be stopped. |
|  | This is the general warning sign. Failure to heed the safety precautions could result in personal injury. |
|  | Avoid shock hazard. If you see either of these symbols, there is a risk of electrical shock in the vicinity. Only qualified persons shall perform the related procedures. |
|  | Avoid fire hazard. Do not test flammable or explosive samples. Read and follow the associated instructions carefully. |
|  | Avoid eye injury. If you see these symbols, there is a risk of exposure to ultraviolet light, which can harm your eyes if safety glasses are not worn. |
|  | Avoid Biohazard. This icon informs of a biological hazard in the area. Read and follow the associated instructions carefully. |
|  | Avoid chemical burns. This symbol alerts you to possible skin irritation. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose of waste. |

| Symbol | Description |
|---|---|
|  | Alternating current |
|  | Earth terminal or ground |
|  | Direct current |
|  | Protective conductor terminal |
|  | Frame or chassis terminal |
|  | Fuse |
|  | Power on |
|  | Power off |

## When the System Arrives

**WARNING** Avoid personal injury. If this equipment is used in a manner not specified in the accompanying documentation, the protection provided by the equipment may be impaired.

**CAUTION** Avoid personal injury. Perform *only* those procedures described in the documentation. If there are other problems, contact us. Any other service must be performed by trained personnel.

**CAUTION** Avoid shock hazard. Do not remove the cover of the instrument. All service to the instrument must be performed by trained personnel.

When the instrument arrives, check the exterior of the shipping box for signs of damage. If damage is apparent, contact us or your local distributor for instructions.

- Move the shipping box to the installation location at least 24 hours before installation.

**Note**

- Inside the shipping box, the instrument is sealed in a plastic bag to keep the unit dry.
- Allow 24 hours for the instrument to reach room temperature before opening the bag. If the bag is opened before the instrument reaches room temperature, moisture could condense on the optical components and cause permanent damage.

- Keep the instrument upright at all times.

The warranty will not cover:

- Damage due to improper moving techniques.
- Damage due to removing the sealed plastic bag before the instrument has come to room temperature.

**Note** It is important to have all system utilities installed before the instrument arrives. Utility installations must comply with all local building and safety codes.

## Lifting or Moving the Instrument

To avoid risk of injury, use proper lifting techniques when lifting or moving the instrument or other system components.

# Electrical Requirements and Safety

Power supplied to the system must be from dedicated, uninterrupted sources. Power must be free of voltage dropouts, transient spikes, frequency shifts, and other line disturbances that impair reliable performance.

If you suspect power quality problems at your site, or if your system will be installed in a heavy industrial environment, we recommend a power quality audit before installation. Contact us or your local electrical authority for more information.

**CAUTION** Avoid shock hazard.

- Only a qualified person using the appropriate measuring device shall check the line voltage, current and frequency.
- Only our trained and certified service representatives shall attempt to service a component that carries this symbol.
- If a protective cover on a system component appears damaged, turn off the system and secure it against any unintended operation. Always examine the protective cover for transport stresses after shipping.
- Even after this instrument has been disconnected from all voltage sources, capacitors may remain charged for up to 30 seconds and can cause an electrical shock.
- Do not allow liquid to run over or into any surface where it may gain entry into the instrument.
- Do not attempt to remove the cover of the instrument.

### Grounding

**CAUTION** Avoid shock hazard. Each wall outlet used must be equipped with a ground. The ground must be a noncurrent-carrying wire connected to earth ground at the main distribution box.

# Power Cords

Be sure to use an appropriate grounded power cord for your electrical service. If the power cord received is not appropriate for the electrical system in your location, or if the power cord becomes damaged, contact us.

### Power Line Conditioning Accessories

A UPS reduces the probability of a system shutdown if power is lost elsewhere in the building. Power line conditioners (which ensure that your service is free from sags, surges or other line disturbances) also are available in the U.S.A. from us for 120 volt operation. Line conditioners for 220 volt operation can be purchased locally. Contact technical support for information about power conditioners and UPS.

## Electrical Service Specifications

The following table lists the specifications for electrical service. Contact our service representative in your area if you have questions about the requirements.

| Requirements | Specifications |
|---|---|
| Input current | 5.0 A (max.) |
| Input voltage | 100-240 VAC |
| Line frequency | 50/60 Hz |
| Line disturbances | Sags, surges or other line disturbances must not exceed 10% of input voltage (even for a half cycle). |
| Noise | < 2 V (common mode)<br>< 20 V (normal mode) |

### Power Consumption

Generally, 50% more power should be available than the entire system (including accessories) typically uses. Maximum power consumption and heat dissipation specifications for the spectrometer and fluorometer and accessories are shown below. The values are approximate.

| Item | Power Consumption | Max. Heat Dissipation |
|---|---|---|
| instrument | 60 W | 205 Btu/hr |

### FCC/IC Notices

This system complies with Part 15 of the FCC Rules and Industry Canada ICES-003 for a Class A system. Operation is subject to the following two conditions:

1. This system may not cause harmful interference, and
2. This system must accept any interference received, including interference that may cause undesired operation.

**Note** This is a Class A product. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.

# Fire Safety and Burn Hazards

**Note** Do not position the instrument so that it is difficult to operate the power switch or access the power supply and power cord.

To avoid a burn injury and the risk of fire or explosion:

- Use caution when testing flammable or explosive samples (see the "Hazardous Materials" section)
- Never block any of the vents on the instrument or its power supply
- Only use exact replacement power supplies from us

# Optical Safety

This instrument was designed with a protective housing to prevent user exposure to ultraviolet light.

# Hazardous Materials

Many standard spectroscopy methods are based on the use of solvents. Others involve corrosive samples or pressurized samples in a gaseous state.

### Volatile Solvents and Flammable Samples

**CAUTION** Avoid personal injury. Do not leave solvents or flammable samples near the instrument. Be sure that the workspace is properly ventilated.

### Compatible Solvents

Most solvents typically used in life science laboratories are compatible with the fiber optic pedestals of all NanoDrop spectrophotometers and fluorometers. However, the high vapor pressure properties of some solvents may not be conducive to small volume measurements when using the pedestal for measurements on any of the NanoDrop instruments. If you are measuring samples with high vapor pressures, use an instrument with provision for measuring samples in cuvettes.

The following solvents are compatible for use on the pedestals of all NanoDrop instruments.

**Note** Spillage of these solvents on surfaces other than the pedestals may damage the instrument.

- methanol
- ethanol
- n-propanol
- isopropanol
- butanol
- acetone
- ether
- chloroform
- carbon tetrachloride
- DMSO
- DMF
- acetonitrile
- THF
- toluene
- hexane
- benzene
- sodium hydroxide
- sodium hypochlorite (bleach)
- dilute HCl
- dilute $HNO_3$
- dilute acetic acid

It is recommended that all corrosive solvents be wiped from the pedestal immediately upon completion of a measurement. It is also recommended that the user end a series of measurements with a di$H_2O$ sample to ensure that solvents are not inadvertently left on the pedestal.

The diaphragm around the pedestal of the NanoDrop is permanently affixed to the instrument. Do not attempt to remove the diaphragm or break the seal. Avoid prolonged exposure of the diaphragm to HCl, alcohol, bleach, acetone or other solvents as the adhesive securing the seal may be affected. If the seal comes loose please contact us.

**Note** All forms of Hydrofluoric Acid (HF) are incompatible as the fluoride ion will etch the fiber optic cable.

## Biohazard or Radioactive Materials and Infectious Agents

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Wear appropriate protective equipment. Individuals should be trained according to applicable regulatory and organization requirements before working with potentially infectious materials. Follow your organization's Biosafety Program protocols for working with and/or handling potentially infectious materials.

Instruments, accessories, components or other associated materials should not be disposed of and may not be returned to us or other accessory manufacturers if they are contaminated with biohazard or radioactive materials, infectious agents, or any other materials and/or conditions that could constitute a health or injury hazard to employees. Contact us if you have questions about decontamination requirements.

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