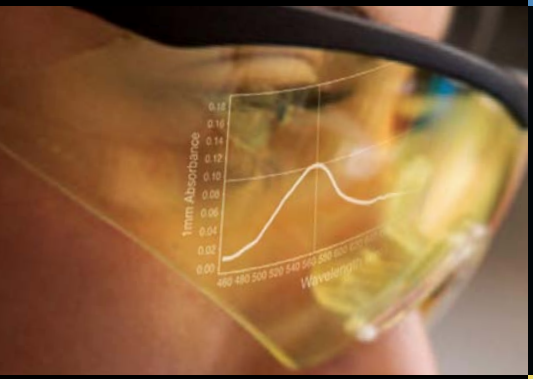
Colorimetric Protein Assays

Thermo Scientific NanoDrop Spectrophotometers



Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

Colorimetric Protein Assays

Revised 11/2010

Contents

Introduction

This booklet is meant to provide some basic protein measurement support information for colorimetric methods relevant to Thermo Scientific NanoDrop 2000/2000c, 8000 and 1000 spectrophotometers. Please refer to the model-specific user manual for more detailed instrument and software feature-related information. Instrument-specific protocols for the four pre-configured methods described in this guide are available under the Science tab at www.nanodrop.com.

Proteins, unlike nucleic acids, can exhibit considerable diversity. The Protein A280 application is appropriate for purified proteins that contain Trp, Tyr residues or Cys-Cys disulphide bonds and exhibit absorbance at 280 nm.

Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry require standard curves and are more commonly used for uncharacterized protein solutions and cell lysates.

Separate booklets for nucleic acid and direct A280 protein measurement methods are also available.

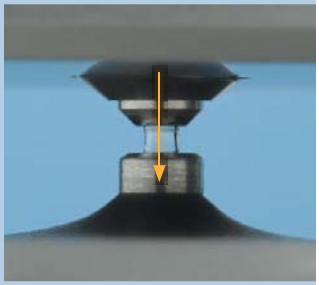
For technical support, please contact:
Thermo Fisher Scientific | NanoDrop Products
3411 Silverside Road | Bancroft Building | Wilmington, DE 19810 USA
Toll-free in US and Canada: 1.877.724.7690
Phone: 1.302.479.7707 | Fax: 1.302.792.7155 | e-mail: nanodrop@thermofisher.com
www.nanodrop.com

Thermo Scientific NanoDrop Instrument Serial Numbers:
NanoDrop 1000: S/N_ _____, S/N_ _____
NanoDrop 2000: S/N_ _____, S/N_ _____
NanoDrop 2000c: S/N_ _____, S/N_ _____
NanoDrop 8000: S/N_ _____, S/N_ _____

Introduction

Sample Retention Technology

- Pipette 1 – 2 µL sample directly onto the measurement pedestal.



- Lower the sampling arm and initiate a spectral measurement using the software on the PC.
- Surface tension is used to hold samples in place between two optical fibers.
- Light from a xenon flashlamp passes through the top optical fiber, down through the liquid column and is detected by the internal spectrometer.

- When the measurement is complete, raise the sampling arm and wipe the sample from both the upper and lower pedestals using a dry, lint-free laboratory wipe.

Best Practices

Cleaning

- An initial cleaning of both measurement surfaces with dH_2O is recommended prior to making the blank measurement. Do NOT use a squirt or spray bottle to apply water or any other liquid to the surface of the instrument.
- Between measurements: Wipe the sample from both the upper and lower pedestals with a clean, dry, lint-free lab wipe.
- A final cleaning of both measurement surfaces with dH_2O is recommended after the last sample measurement. Do NOT use a squirt or spray bottle to apply water or any other liquid to the surface of the instrument.
- Additional cleaning: Use 3 µL of HCl instead of the dH_2O for cleaning when samples have dried on the pedestal. Follow with a 3 µL aliquot of dH_2O.
- Detergents and isopropyl alcohol are NOT recommended cleaning agents as they may uncondition the pedestal measurement surfaces. If a solution containing detergents or alcohol is used, follow with 3 – 5 µL of dH_2O.

Reconditioning

Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the hydrophobic surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
2. Apply a very thin, even layer of PR-1 to the flat surface of the upper and lower pedestals. Wait 30 seconds for the PR-1 to dry.
3. Fold a clean, dry, lint-free lab wipe into quarters and remove the PR-1 by rubbing the surface of the upper and lower pedestals until no additional dark compound residue shows on the lab wipe.

Pedestal Assessment

Droplet "flattens out" on unconditioned pedestal

Droplet "beads up" on properly conditioned pedestal

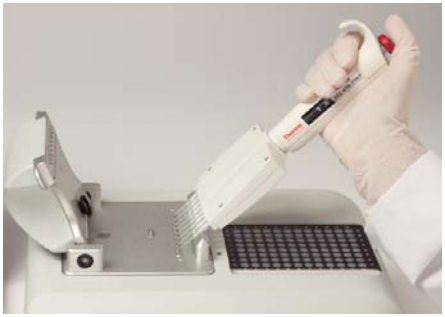
To check the effectiveness of the reconditioning, pipet a 1 µL aliquot of dH_2O onto the lower measurement pedestal and visually verify that the water "beads up."

Best Practices
Instrument Orientation

Angle the instrument for optimal pipetting.

Right-handed orientation for the NanoDrop 2000/2000c and close-up view of the NanoDrop 8000.

Below, recommended orientation for left- and right-handed sample dispensing on the NanoDrop 8000.



Cuvette Orientation (NanoDrop 2000c only)

Use etched arrow as light path guide when inserting quartz or masked plastic cuvette.

Tip: Locate instrument away from air currents and exhaust fans.

Best Practices

Sampling Technique

Volume Requirement Use adequate sample volume to ensure good column formation.

Tip: Always use 2 µL samples when measuring protein samples to ensure proper column formation. Surfactants and other components routinely used in protein buffers may reduce the surface tension properties of the liquid.

Pipettor Selection Use calibrated pipettor with well-fitting tips.

It is best to use a precision pipettor (0 – 2 µL) with low retention precision tips to ensure that sufficient sample (2 µL) is delivered for optimal column formation.

*Tip: To avoid evaporation errors, it is essential to use an eight-channel pipettor to **simultaneously** load samples when using two or more pedestal positions on the NanoDrop 8000.*

Sample Aliquots **Always** use fresh tips and fresh aliquots for every measurement.

Tip: Repeated measurements on the same sample aliquot will result in evaporation, yielding increasing concentrations and/or column breakage.

Sample Homogeneity Highly concentrated protein samples require careful attention to ensure homogeneity before sampling.

Tip: Non-reproducible results observed when making small volume measurements are a good indicator that the sample is not well mixed.

Sample Preparation Follow assay reagent manufacturer's recommendations for optimal sample preparation.

Colorimetric Assays

Colorimetric assays such as the Pierce 660 nm, BCA, Bradford and Lowry are generally used for uncharacterized protein solutions and cell lysates.

Protein standards, pre-diluted standards and protein purification products are available from Thermo Fisher Scientific at the following website: www.piercenet.com.

The information below is meant to be a useful guide for determining which assay to use. Refer to the reagent or kit manufacturer for more information concerning buffer compatibility and assay optimization.

Method	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility (minimum 5 replicates) (SD = µg/mL; CV = %)
Pierce 660 nm	50 µg/mL (15:1 reagent/sample volume)	2000 µg/mL	50 – 125 µg/mL: ± 3 µg/mL >125 µg/mL: ± 2%
	25 µg/mL (7.5:1 reagent/sample volume)	1000 µg/mL	25 – 125 µg/mL: ± 3 µg/mL >125 µg/mL: ± 2%
BCA	200 µg/mL (20:1 reagent/sample volume)	8000 µg/mL	± 2% (over entire range)
	10 µg/mL (1:1 reagent/sample volume)	200 µg/mL	± 10 µg/mL (over entire range)
Bradford	100 µg/mL (50:1 reagent/sample volume)	8000 µg/mL	100 – 500 µg/mL: ± 25 µg/mL 500 – 8000 µg/mL: ± 5%
	15 µg/mL (1:1 reagent/sample volume)	100 µg/mL	15 – 50 µg/mL: ± 4 µg/mL 50 – 125 µg/mL: ± 5%
Modified Lowry	200 µg/mL (5:1 reagent/sample volume)	4000 µg/mL	± 2% (over entire range)

Colorimetric Assays

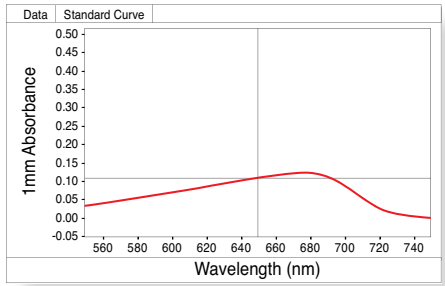
Pierce 660 nm Protein Assay

This assay is based on a dye-metal complex that binds to proteins in an acidic solution. Upon binding, the reddish dye-metal complex changes to green, resulting in an absorbance that is measured at 660 nm and normalized at 750 nm.

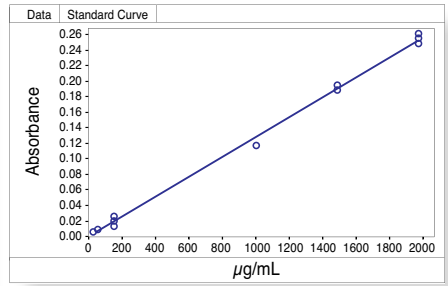
Advantages:

- Room temperature stability of the assay reagent.
- A greater linear range than the Coomassie-based Bradford assays.
- Compatibility with commonly used detergents and reducing agents.
- Compatibility with samples lysed in Laemmli sample buffer.
- Rapid mix-and-read protocol.

This assay can quantify peptides that are at least 2,500 daltons if compositions include amino acid residues that react with the dye-metal reagent (i.e., histidine, arginine, tyrosine, tryptophan and phenylalanine).



Typical Pierce 660 nm Sample Spectrum



Typical Pierce 660 nm Standard Curve

The micro-assay has a linear range of 25 – 1000 µg/mL for BSA using a 7.5:1 reagent to sample ratio. A higher range of 50-2000 µg/mL for BSA may be obtained using a 15:1 reagent to sample ratio.

The data presented above were generated using a 15:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 4 µL into 56 µL of reagent for a total volume of 60 µL is recommended.

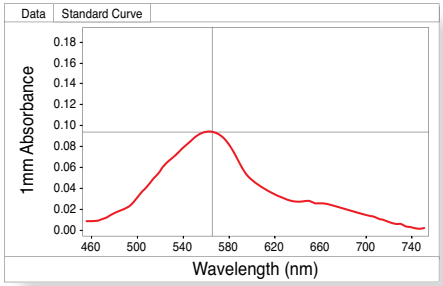
Colorimetric Assays

BCA (Bicinchoninic Acid) Protein Assay

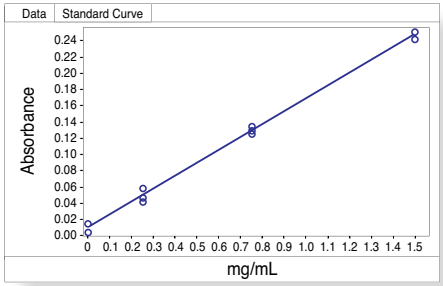
This assay uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm and normalized at 750 nm.

Advantages:

- Exhibits less protein-to-protein variation than dye-binding methods.
- Compatible with typical concentrations of most ionic and nonionic detergents.
- Much easier and four times faster than the classical Lowry method.



Typical BCA Sample Spectrum



Typical BCA Standard Curve

The micro-assay has a linear range of 20 – 125 µg/mL for BSA using a 1:1 reagent to sample ratio. A higher range of 125-2000 µg/mL for BSA may be obtained using a 20:1 reagent to sample ratio.

The data presented above were generated using a 20:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 5 µL into 95 µL of reagent for a total volume of 100 µL is recommended.

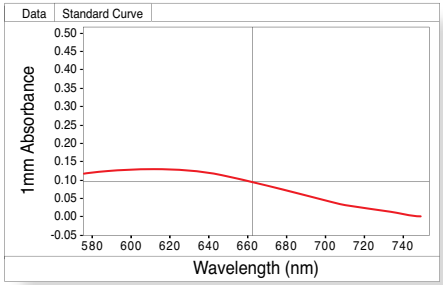
Colorimetric Assays

Bradford Protein Assay

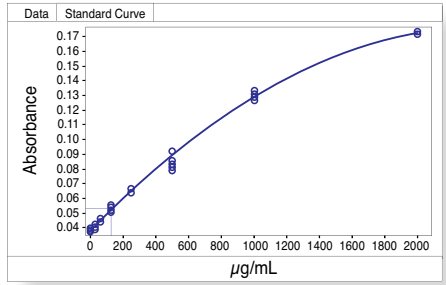
This assay uses a protein-induced absorbance shift of Coomassie Blue dye as a measure of protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm.

Advantages:

- Single reagent; no working reagent preparation required.
- Fast with almost immediate color development; add, mix and read results.



Typical Bradford Sample Spectrum



Typical Bradford Standard Curve

The micro-assay has a linear range of 15 – 100 µg/mL for BSA using a 1:1 reagent to sample ratio. A higher range of 100 – 8000 µg/mL for BSA may be obtained using a 50:1 reagent to sample ratio. Note: The best linearity is in the 100 – 1000 µg/mL range.

The data presented above were generated using a 50:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 4 µL in 196 µL of reagent for a total of 200 µL is recommended.

Bradford Protein Assay Considerations

- Allowing the reaction to incubate for a longer than suggested time frame increases the potential for interfering aggregates.
- Higher protein concentrations may increase the possibility of dye or dye-protein aggregates contributing to interfering light scattering.
- The use of Bradford assay reagents may result in unconditioned pedestals over time. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the hydrophobic surface properties have been compromised and liquid columns break during measurement.

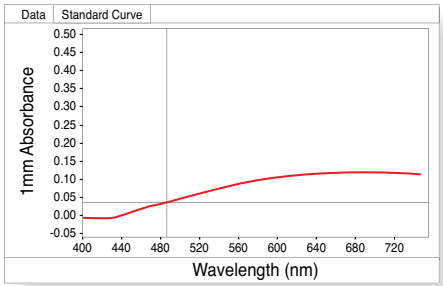
Colorimetric Assays

Modified Lowry Protein Assay

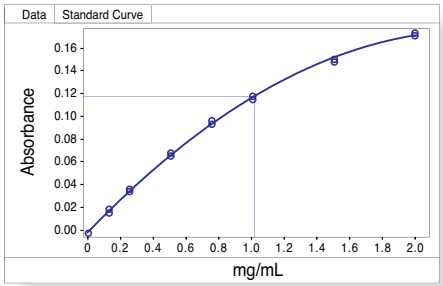
This assay is based on the widely used and cited Lowry procedure for protein quantitation. The procedure involves a reaction of protein with cupric sulfate in alkaline solution, resulting in formation of tetradentate copper-protein complexes that is measured at 650 nm on NanoDrop spectrophotometers and normalized at 405 nm.

Advantages:

- Widely cited in protein research literature.
- Modified cupric sulfate-tartrate reagent is stable at room temperature.



Typical Modified Lowry Sample Spectrum



Typical Modified Lowry Standard Curve

The Modified Lowry has a linear range from ~ 200 µg/mL up to 4000 µg/mL using a 5:1 reagent to sample ratio.

The data presented above were generated using a 5:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 10 µL into 40 µL of reagent for a total volume of 50 µL is recommended.

Colorimetric Assays Considerations

- Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring both are subjected to the same timing and temperature throughout the assay.
- Assay protocols for colorimetric assays specific for the NanoDrop 2000/2000c and the NanoDrop 8000 are available under the Science tab at www.nanodrop.com.

Measurements

Basic Protocol

Refer to the manufacturer's guidelines and recommendations for sample preparation.

There may be signal output differences due to the change in pH when using diluents such as dH_2O and a neutral pH PBS buffer. Be sure to use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.

1. A standard curve is required every time an assay is run.

   Tip: Although the operating software allows for curves to be saved and reloaded, it is recommended that the user follow the manufacturer's guidelines and generate a new standard curve each time the assay is run.

2. Follow prompts to initialize the spectrometer.

3. After initialization, use a dry, lint-free lab wipe to remove the water from the upper and lower pedestals. (NanoDrop 1000 and NanoDrop 8000 only.)

4. Enter the values for each standard. The operating software allows for the zero reference and up to 7 additional standards. Fewer standards may be run at the user's discretion.

   Tip: The appropriate blank for most colorimetric assays is dH_2O. However, for the Pierce 660 nm Protein Assay, the diluent (no protein) plus dye reagent solution is used as both the blank and the zero reference.

5. Apply 2 µL of dH_2O to the lower pedestal, lower the upper arm and select **Blank**.

   Tip: A zero reference is the diluent (no protein) plus dye reagent. The minimum requirement for standard curve generation is the zero reference plus one standard or two standards. The NanoDrop software will not extrapolate concentrations beyond the defined standard curve.

   Tip: If using more than one pedestal position on the NanoDrop 8000, it is important to use a multi-channel pipettor to deliver the standard or sample aliquots.

6. Using a dry, lint-free laboratory wipe, remove the blank solution from the upper and lower pedestals.
7. Select the zero reference, place 2 µL of the reference solution on each lower pedestal, lower the upper arm and select **Measure**.
8. Using a dry, lint-free laboratory wipe, wipe the reference solution from the upper and lower pedestals.
9. Select and then measure the next standard. Up to 5 replicate measurements can be made for each standard.

Tip: If measuring multiple replicates of the same standard or sample, it is important to use a fresh aliquot for every measurement.

10. Review the standard curve and choose the appropriate curve fit.
11. After the standard curve is complete, enter the sample name in the Sample ID box and begin measuring the samples.
12. Pipette an aliquot of the protein sample onto the lower measurement pedestal and lower the sampling arm. Click **Measure**.

Tip: It is important to indicate whether a sample should be included in a workbook or report prior to the measurement. Refer to the instrument-specific user manual for instructions on including sample measurement information to a report or workbook.

Lab Notes:

Calculations

Standard Curves

- Prepare both standards and unknowns in the same manner. Be sure to use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- A standard curve can be generated using a minimum of two points which may include two standards or a reference (reagent/assay buffer only—no protein) and one standard.
- The multi-point curve capability allows for multiple replicates for up to 7 standards. There is no set order in which standards must be run.
- Standards diluted from a stock standard must cover the expected range of the unknown samples. Pre-diluted standard sets may be available from some manufacturers.
- Although standard curves may be saved, it is recommended that a new standard curve be generated each time the assay is run.

Refer to the table below for assay-specific standard curve type recommendations:

Method	Reagent Volume/ Sample Volume	~ Detection Limit	Suggested Curve Type
Pierce 660 nm	15 to 1	50 µg/mL to 2000 µg/mL	Linear
	7.5 to 1	25 µg/mL to 1000 µg/mL	Linear
BCA	20 to 1	200 µg/mL to 8000 µg/mL	Linear
	1 to 1	10 µg/mL to 200 µg/mL	Linear
Bradford	50 to 1	100 µg/mL to 8000 µg/mL	2nd order polynomial
	1 to 1	15 µg/mL to 100 µg/mL	2nd order polynomial
Modified Lowry	5 to 1	200 µg/mL to 4000 µg/mL	2nd order polynomial

Calibration

Calibration Verification

All NanoDrop spectrophotometers include a diagnostic application which allows the user to run a Calibration Check procedure to confirm that the instrument is working within specifications.

Wavelength Calibration (Automatic)

- Wavelength calibrations using standard reference lines in the xenon flashlamp spectrum are automatically performed within the operating software.
- This verification ensures wavelength accuracy and does not require any action by the user.

Pathlength Verification (User-performed)

- Use CF-1 in conjunction with the Calibration Check diagnostic to verify that the pathlengths are within specification.
- The pathlengths used to make measurements are the same across all wavelengths. Therefore, when pathlengths are verified at one wavelength, the verification is valid for the entire measured spectrum.

Calibration Check Fluid

- CF-1 is a standard manufactured exclusively for use with NanoDrop Spectrophotometers and is available from Thermo Fisher Scientific and its distributors.
- The CF-8 Calibration Kit (used for the NanoDrop 8000 calibration check procedure) includes 2 CF-1 vials as well as 8-well PCR strip tubes.

Tip: It is good practice to check the instrument's performance every six months with a new vial of NanoDrop Calibration Check Fluid.

Calibration

Standard vs Control

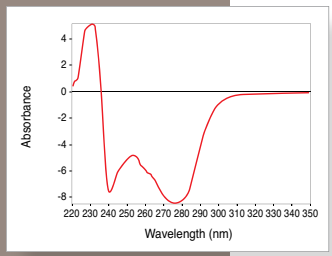
A "Standard" is generally accepted as a solution of a **known concentration** that is used to calibrate or certify that an instrument is working within acceptable, pre-defined guidelines.	• The NanoDrop CF-1 Calibration Check Fluid is the only acceptable standard for use with the NanoDrop instrument Calibration Check diagnostic available within the operating software. • The term "Standard" also refers to protein solutions of a known concentration used to define a standard curve. These standards are not appropriate to assess NanoDrop instrument performance.
A "Control" is a solution that produces an expected result within a **specific range** if the "system" is working as expected. The definition of system would include the instrument, protocols being used, techniques employed by the user and the solution utilized as the control.	• 2 µg/mL Bovine Serum Albumin solution is a routine laboratory control used to monitor the reproducibility and values obtained from day to day use. • Controls are valid to use as long as the instrument is calibrated and the control product itself is within the expected concentration range stated in the manufacturer's specifications.

Tip: Ensure all controls are stored as recommend by the manufacturer. Do not use controls past the stated expiration date.

Troubleshooting

Unusual Spectra

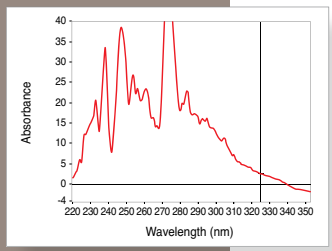
When troubleshooting sample measurements, it is important to utilize the sample spectrum as a primary guide.



Negative values associated with some spectra indicate that either the pedestals were very dirty when the blank measurement was made or that a sample was used to make a blank or reblank measurement.

Suggestion:

Clean pedestal and measure new blank.

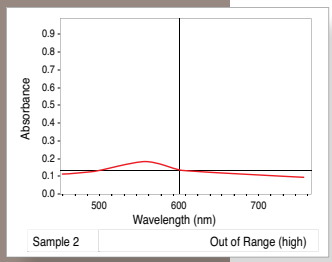


A jagged appearance throughout a spectrum may indicate a broken column.

Suggestions:

Clean pedestal and recondition the pedestal surfaces and measure new blank.

Increase sample volume to ensure proper column formation.



A reported concentration value of NaN or Out of Range indicates that the sample concentration is outside of the range of the measured standard curve.

Suggestion:

Always include standards that will cover the expected range of the samples prior to sample measurement.

Troubleshooting

Reproducibility

Non-reproducible results are usually due to issues with sample non-homogeneity, blanking on a dirty pedestal, using the same aliquot for multiple measurements, or column breakage.

Sample Heterogeneity

- Sampling from non-homogeneous solutions, particularly when using small volumes, may result in significant measurement deviation in the generated data.
- Ensure sample and assay reagent are well mixed.

Tip: Prolonged incubation with the Bradford reagent may result in the formation of particulates.

Dirty Pedestal

- Clean and recondition the pedestal surfaces prior to the start of the measurement session.

Multiple Measurements

- Use fresh aliquots for each pedestal measurement.

Tip: Multiple measurements of the same aliquot will result in evaporation and increased sample concentration values.

Column Breakage

- Visually check that a column is intact after the completion of the measurement. If not, refer to the *Instrument Related Issues* section for guidance.

Troubleshooting

Reproducibility

Concentrations not within Expected Range

- Confirm that your sample is not too dilute or too concentrated.

  Tip: Refer to the table of method-specific detection limits on page 9 for guidance.

- Confirm that the reference (blank) solution and sample diluent are of the same composition, ionic strength and pH.
- Ensure no unnecessary dilutions are being factored into the assay.

Instrument Related Issues

Column Breakage

- Ensure pedestal surfaces are properly conditioned.

  Tip: When a pedestal becomes unconditioned, sample droplets applied to the bottom pedestal will "flatten out" and cover the entire pedestal surface rather than "bead up." Refer to the Reconditioning instructions under the ***Best Practices*** *section on page 6.*

- Ensure sufficient volume is loaded onto the pedestal.
- Use a larger volume (1.5 – 2 µL) for each measurement.
- Use a calibrated small volume pipettor to deliver the sample to the pedestal.
- Ensure instrument is not located near a vent or other source of air flow.
- Ensure measurements are made immediately after pipetting samples onto the pedestal, as delays may compromise accuracy.
- If an error message indicating possible column breakage is displayed and the user visually confirms that the liquid column is forming, perform a calibration check. If the instrument is out of calibration, contact **Technical Support**. Outside of the US and Canada, please contact your local NanoDrop products distributor.

Troubleshooting

Installation Errors

Usually, an installation failure is the result of an unsuccessful installation of the device drivers.

- Verify system specifications meets published requirements.
- Verify that user has full Administrator access to the software and data folders and that the use of USB devices is acceptable.
- Verify that the instrument is receiving power.
- Verify driver installation using the Device Manager:
  1. Locate the **My Computer** icon on the desktop or access through the Windows Start menu. Right click on **My Computer**.
  2. Highlight and select **Manage**.
  3. Click on **Device Manager** in the left pane.
  4. Locate the NanoDrop device folder from the list displayed in the right pane and click on the **+** *(plus sign)* to open.

     Tip: Yellow exclamation points or question marks associated with either a NanoDrop or an unknown device indicate drivers did not install properly.

  5. Highlight and delete the questionable device.
  6. Unplug the USB cable from the computer and the power cord from the instrument. Wait 10 seconds, then reconnect beginning with the power cord.

If the error persists, contact **Technical Support**. Outside of the US and Canada, please contact your local NanoDrop products distributor.

Troubleshooting

Connection Errors

If your instrument operates properly most of the time, but connection errors appear intermittently, the instrument may not be receiving power or recognizing the USB connection.

- Ensure that the USB and power cables are plugged into the back of the instrument, and that the instrument is receiving power.
- Many instrument issues can be addressed by a simple power restart.
  1. Exit the software.
  2. Disconnect the instrument power cord and USB cable.
  3. Reconnect the instrument power cord first, then the USB cable.
  4. Restart the software.

If the error persists, contact **Technical Support**. Outside of the US and Canada, please contact your local NanoDrop products distributor.

Signal Errors

Some error messages are triggered when little to no light reaches the detector during initialization or a measurement.

- Refer to the cleaning directions under the ***Best Practices*** section on page 6.
- Run the Intensity Check diagnostic. Refer to the model-specific user guide for additional information.

FAQs

Q: What are the sample size requirements when using NanoDrop spectrophotometers?

A: We recommend using a 2 µL sample size for pedestal-based protein measurements. Proteins and/or protein buffers may alter the surface tension properties of the solution and using the larger sample size is recommended to ensure proper column formation.

Q: What sort of accuracy should I expect with NanoDrop spectrophotometers?

A: Typically within 2%.

Q: What sort of reproducibility should I expect with NanoDrop spectrophotometers?

A: See chart below.

Method	Lower Detection Limit	Typical Reproducibility (minimum 5 replicates) (SD = µg/mL; CV = %)
Pierce 660 nm	50 µg/mL to 2000 µg/mL (15:1 reagent/sample volume)	50 – 125 µg/mL: ± 3 µg/mL >125 µg/mL: ± 2%
	25 µg/mL to 1000 µg/mL (7.5:1 reagent/sample volume)	25 – 125 µg/mL: ± 3 µg/mL >125 µg/mL: ± 2%
BCA	200 µg/mL to 8000 µg/mL (20:1 reagent/sample volume)	± 2% (over entire range)
	10 µg/mL to 200 µg/mL (1:1 reagent/sample volume)	± 10 µg/mL (over entire range)
Bradford	100 µg/mL to 8000 µg/mL (50:1 reagent/sample volume)	100 – 500 µg/mL: ± 25 µg/mL 500 – 8000 µg/mL: ± 5%
	15 µg/mL to 100 µg/mL (1:1 reagent/sample volume)	15 – 50 µg/mL: ± 4 µg/mL 50 – 125 µg/mL: ± 5%
Modified Lowry	200 µg/mL to 4000 µg/mL (5:1 reagent/sample volume)	± 2% (over entire range)

FAQs

Q: Can I save and reuse a standard curve?

A: Yes, although the recommendation is to generate a new standard curve each time a set of samples is to be measured. Refer to the assay reagent manufacturer for additional information.

Q: Will the software extrapolate protein concentrations for samples outside of the measured standard curve?

A: No. It is important to run a range of standards that will cover the full range of the expected sample concentrations. Some assay reagents may not yield a linear response for higher concentration samples. Refer to the assay reagent manufacturer for additional information.

Q: Is simply wiping the pedestal surface enough to prevent carryover?

A: Yes. The highly polished quartz and stainless steel surfaces of the sample retention system are resistant to sample adherence, making the use of dry, lint-free lab wipes very effective in removing the sample.

Q: How do I keep my sample from flattening out on the measurement pedestal?

A: Use the NanoDrop PR-1 Reconditioning Compound as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. PR-1 kits are available through Thermo Fisher Scientific or your local distributor.

Q: What is the cause of negative absorbance values?

A: A blank measurement was made either using a solution with more absorbance than the sample of interest or on a dirty pedestal. Clean the pedestal and make a new blank measurement with a fresh aliquot of the appropriate buffer.

Q: How do I check the accuracy of NanoDrop spectrophotometers?

A: NanoDrop CF-1 Calibration Check fluid should be used with the Calibration Check diagnostic in the instrument software. CF-1 is prepared from the NIST potassium dichromate standard SRM935 in acidified reagent grade water.

FAQs

Q: How do I calibrate NanoDrop spectrophotometers?

A: The calibration check procedure allows the user to confirm that the instrument is performing within specifications. If the instrument requires recalibration, contact **Technical Support**. Outside of the US and Canada, please contact your local NanoDrop products distributor.

Q: Where is the data stored?

A: The NanoDrop 2000/2000c software allows the user to designate a workbook (.twbk) at a location of the user's preference for recording measurements. The default data storage location is in the ***My Documents*** folder. The NanoDrop 8000 and the NanoDrop 1000 models automatically archive all measurement data in a folder on the C drive. Refer to the model-specific user manual for additional details. A PDF of each manual may be found at www.nanodrop.com.

Q: Is the flash lamp continuously on, or is it on only when performing a measurement?

A: The lamp is on only during measurements.

Q: Are there solvent restrictions?

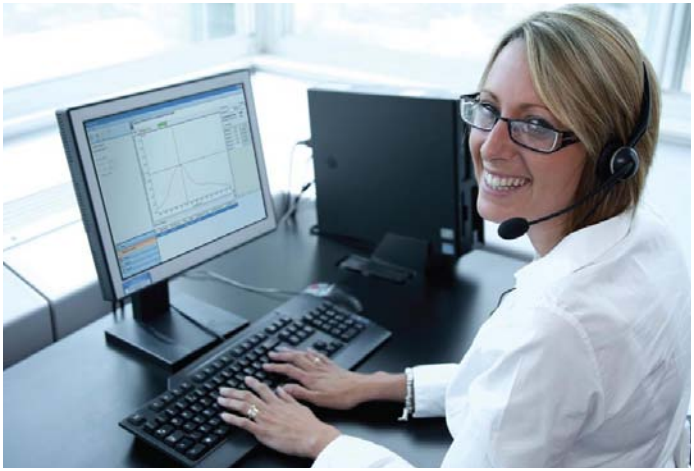
A: Yes. Do not use hydrofluoric acid on the pedestal as it may etch the quartz optical fiber. Most other laboratory solvents typically used in life science labs, including dilute acids, are compatible with the pedestal as long as they are immediately wiped off upon the completion of the measurement.

Tip: The use of volatile solvents for sample measurement may result in erroneous data due to the rapid evaporation of the 1 – 2 µL sample volume.

Technical Support

For additional assistance, please contact us at **1.877.724.7690** or send an email to: nanodrop@thermofisher.com. The Thermo Scientific NanoDrop Product Technical Support Team is available between 9am and 5pm, EST.

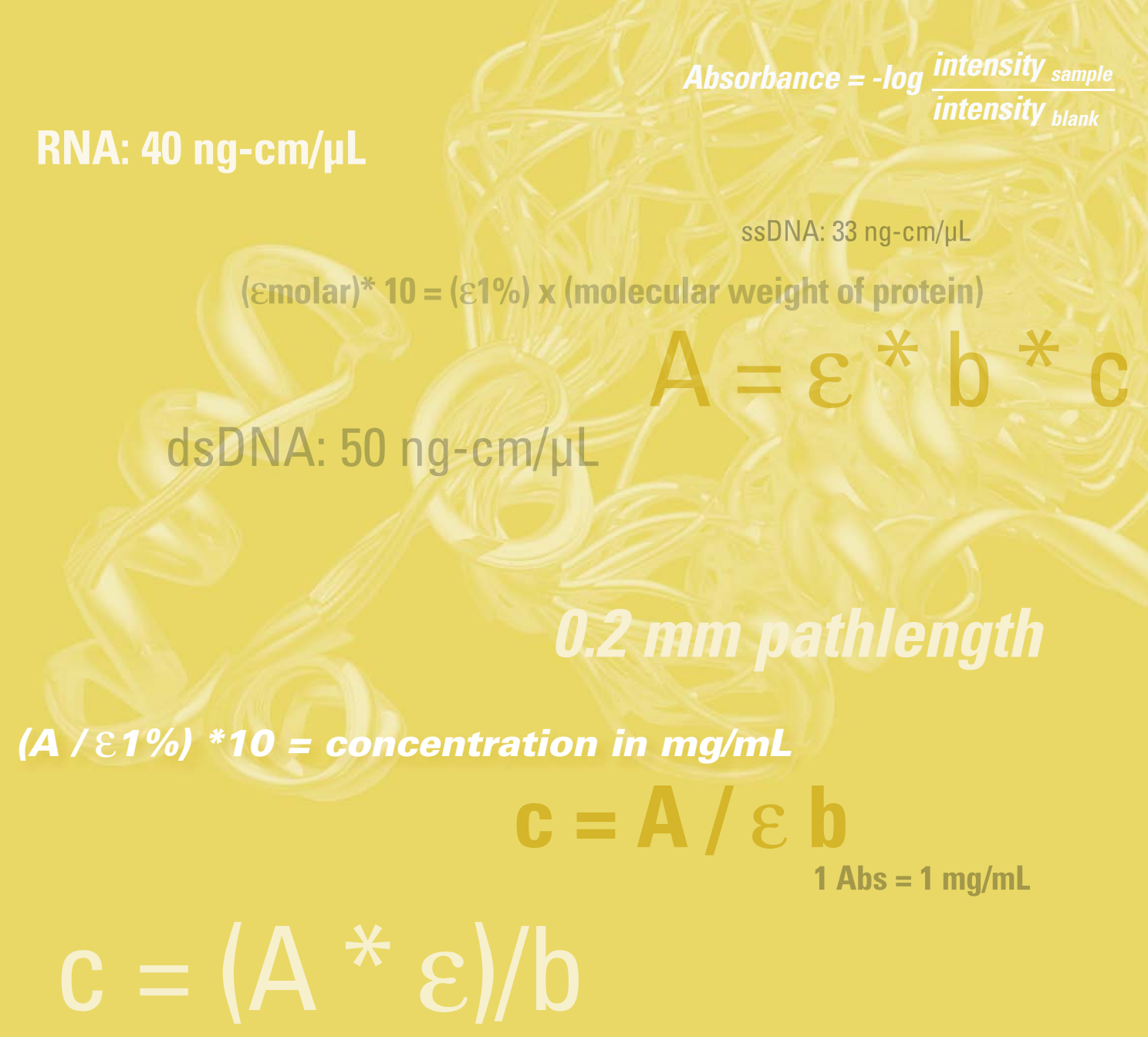
For technical support outside of the US and Canada, please contact your local Thermo Scientific NanoDrop products distributor.



Additional technical information is available at: **www.nanodrop.com.**

Thermo Fisher Scientific | NanoDrop Products
3411 Silverside Road | Bancroft Building
Wilmington, DE 19810 U.S.A.
Toll-free in US and Canada: 1.877.724.7690
Phone: 1.302.479.7707
Fax: 1.302.792.7155
E-mail: nanodrop@thermofisher.com
www.nanodrop.com

Additional Notes:


Absorbance = -log intensity sample / intensity blank
RNA: 40 ng-cm/µL
ssDNA: 33 ng-cm/µL
(εmolar)* 10 = (ε1%) x (molecular weight of protein)
A = ε * b * c
dsDNA: 50 ng-cm/µL
0.2 mm pathlength
(A / ε1%) *10 = concentration in mg/mL
c = A / ε b
1 Abs = 1 mg/mL
c = (A * ε)/b

Thermo Scientific NanoDrop Spectrophotometers
Colorimetric Protein Assays

Thermo Fisher Scientific | NanoDrop Products
3411 Silverside Road, Bancroft Building
Wilmington, DE 19810 USA

www.nanodrop.com
1.877.724.7690 | 1.302.479.7707

T106 Rev. 11/2010

Thermo SCIENTIFIC