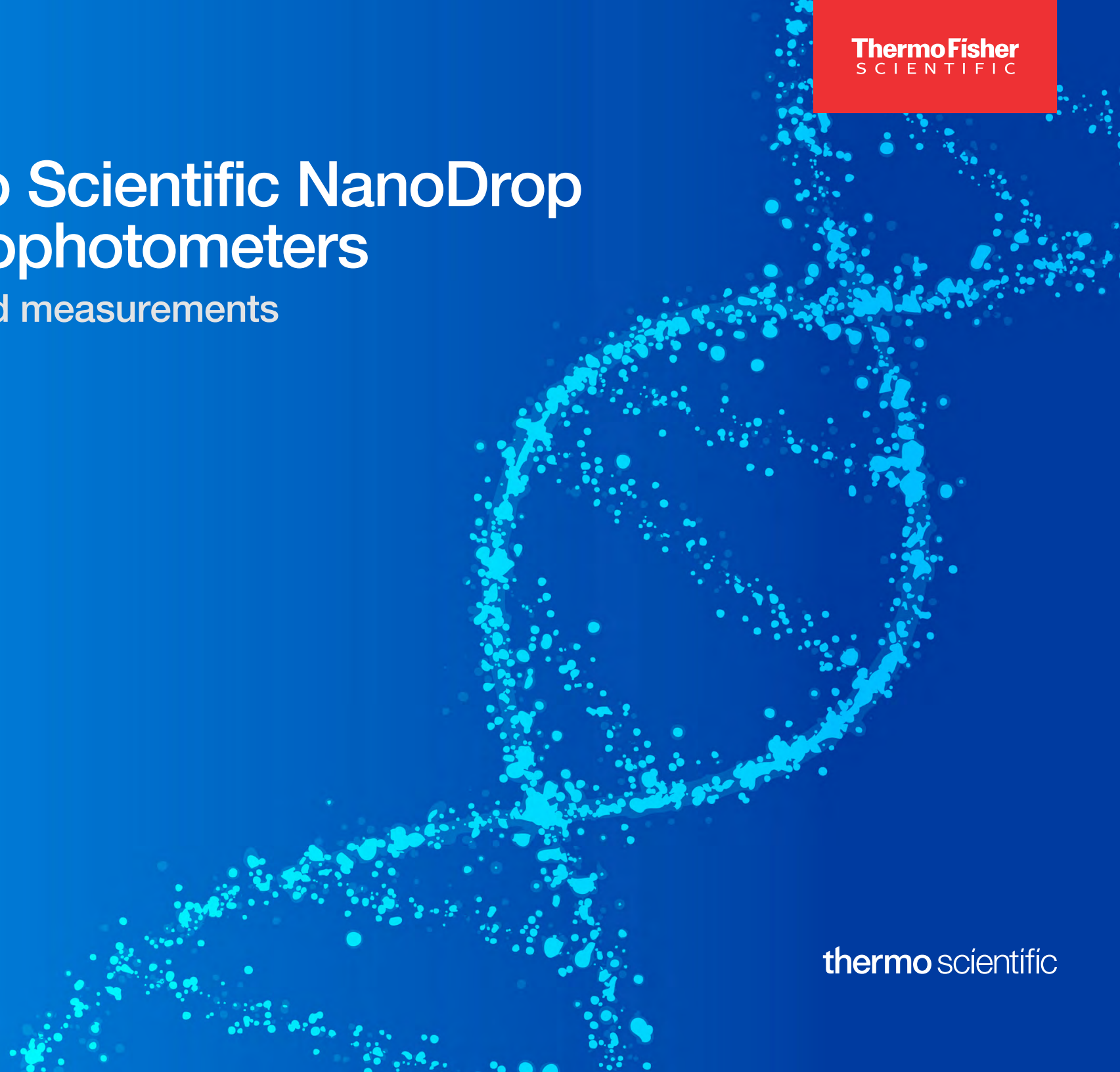
ThermoFisher SCIENTIFIC

Thermo Scientific NanoDrop Spectrophotometers

Nucleic acid measurements



thermo scientific

Contents

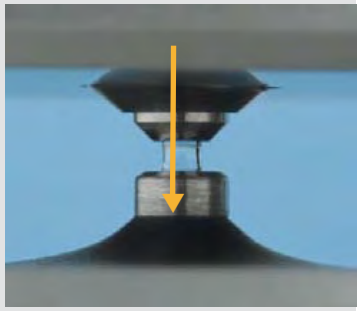
Introduction

This booklet provides nucleic acid measurement support information relevant to Thermo Scientific™ NanoDrop™ One/One^{C}, Eight, and Lite Plus Spectrophotometers. Please refer to the model-specific user manuals for more detailed instrument and software feature-related information.

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

Sample retention technology

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



- Lower the sampling arm and initiate a spectral measurement.
- Surface tension is used to hold samples in place between two optical fibers.
- Light from a xenon flashlamp passes through the top optical fiber and down through the liquid column, where it 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.

Using this technology, NanoDrop Spectrophotometers have the capability to measure samples between 50 and 200 times more concentrated than samples measured using a standard 1 cm cuvette.

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 0.5M 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 laboratory 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, pipette a 1 µL aliquot of dH_2O onto the lower measurement pedestal and visually verify that the water “beads up.”

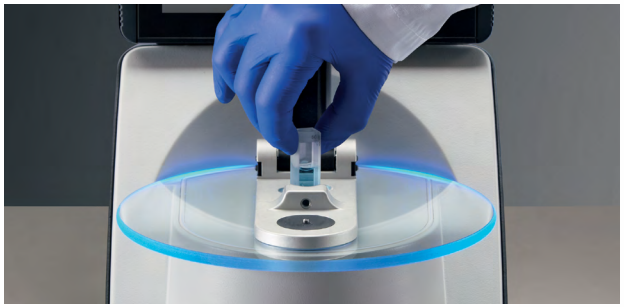
Instrument orientation

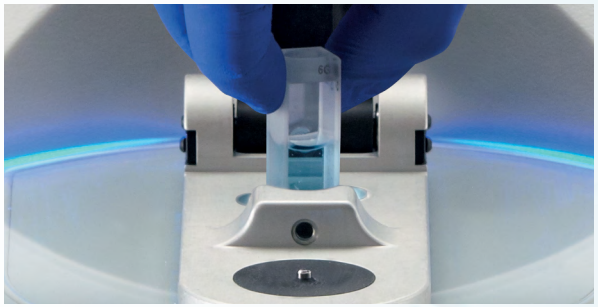
Angle the instrument for optimal pipetting.

Shown here is the right-handed orientation for the NanoDrop One/One^{C} (the left-handed orientation is equally viable if preferred) and a close-up view of the NanoDrop Eight.

Recommended orientation for left- and right-handed sample dispensing on the NanoDrop Eight. The magnetic pipette guide can be moved to support preferred handedness.

Cuvette orientation (NanoDrop One^{C} 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.

Sampling technique

Volume requirement : Use adequate sample volume to ensure good column formation.

Tip: Although 1 µL volumes are usually sufficient for most sample measurements, increasing the sample size to 2 µL will ensure proper column formation for samples with reduced surface tension.

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 (1 – 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 Eight Spectrophotometer.

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 nucleic acid 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 fully in solution or is not homogenous.

Tip: It may be necessary to heat and/or lightly vortex samples prior to sampling to ensure homogeneity.

Sample preparation and purification

Sample preparation: Ensure sample isolation procedure is optimized and sample is purified prior to measurement.

Any molecule that absorbs at 260 nm will contribute to the total absorbance value used to calculate sample concentrations. Examples include DNA, RNA, free nucleotides, proteins and some dyes, as well as many other molecules found in common buffers.

Tip: To minimize the potential of overestimating a nucleic acid sample concentration as a result of an interfering substance, it is important to purify samples prior to making absorbance measurements.

Lab notes

Blank measurements

1. Perform the Cleaning and Reconditioning procedures outlined in the Best Practices section on page 5.
2. Open the Nucleic Acids tab and select the application corresponding to the desired sample type.

Sample type	Constant used to calculate concentration	Instrument availability
dsDNA	50	All
ssDNA	33	All
RNA	40	All
Oligo	Oligo calculator defined	NanoDrop One/One^{C} and NanoDrop Eight
Custom factor	15-150*	NanoDrop One/One^{C} and NanoDrop Eight

* Range for user-entered constant.

3. Load 1-2 µL of the blanking buffer onto the pedestal surface and lower the arm. If **Auto-Blank** is On, the blank measurement starts automatically after the arm is lowered (not available on the NanoDrop Lite Plus). Click or Tap **Blank** if Auto-Blank is not activated.

> **Tip:** The blank solution should be the same pH and of a similar ionic strength as the sample solution. For nucleic acid samples, blank buffers are generally dH_2O or TE. Blanking with water for samples dissolved in TE may result in low 260/230 ratios.

4. After the measurement is complete, use a dry, lint-free lab wipe to remove the buffer from both the top and bottom pedestal surfaces.
5. It is best practice to run a blanking cycle after a blank is performed as follows: Pipette 1-2 µL of blank solution onto the pedestal and lower the arm. If **Auto-Measure** is On, the measurement starts automatically after the arm is lowered (not available on the NanoDrop Lite Plus). Click or Tap **Measure** if Auto-Measure is not activated.

> **Tip:** The result should be a spectrum that varies no more than 0.04 Abs (10 mm absorbance equivalent) from the baseline at 260 nm. If not, clean the measurement surfaces and repeat steps 3–5. Although it is not necessary to blank between each sample, it is recommended that a new blank be taken every 30 minutes when measuring many samples.

Sample measurements

2. Pipette an aliquot of the nucleic acid sample onto the lower measurement pedestal and lower the arm. Click or Tap **Measure** if Auto-Measure is not activated.

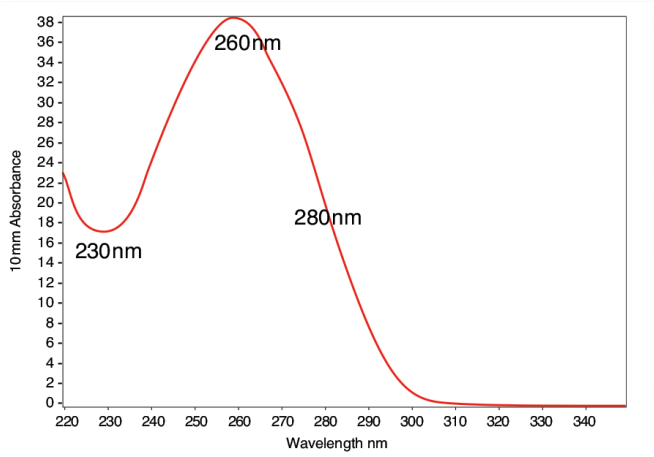
> **Tip:** If measuring more than one sample at a time on the NanoDrop Eight Spectrophotometer, it is important to use a multi-channel pipettor to deliver the sample aliquots.

3. After the measurement is complete, use a dry, lint-free lab wipe to remove the sample from both the top and bottom measurement surfaces.

4. Change pipette tips for the next measurement.

> **Tip:** If measuring multiple replicates of the same sample, it is important to use a fresh aliquot for every measurement to ensure accurate results.

5. Review spectral image to assess sample quality.



Typical Nucleic Acid Spectrum

Refer to the *Purity Ratios* and *Troubleshooting* sections on pages 13 and 14 for more information.

Concentration calculations

Modified Beer-Lambert equation

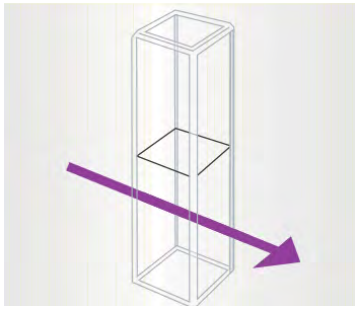
For nucleic acid quantification, a modification of the Beer-Lambert equation is used to calculate sample concentrations.	$c=(A * \varepsilon)/b$ • c is the nucleic acid concentration in ng/µL • A is the absorbance in AU • ε is the wavelength-dependent extinction coefficient in ng-cm/µL • b is the pathlength in cm
Nucleic acid sample concentrations are based on the absorbance at 260 nm, the selected analysis constant and a baseline correction.	• The correction normalizes for any baseline offset attributable to light scattering artifacts. • The wavelength for the baseline normalization is 340 nm. **Tip:** The user may manually enter a different wavelength to be used for the baseline normalization of nucleic acid samples when using the NanoDrop One/One^{C} or NanoDrop Eight Spectrophotometer.
The generally accepted extinction coefficients or constants for nucleic acids are as indicated to the right:	• Double-stranded DNA: 50 ng-cm/µL • Single-stranded DNA: 33 ng-cm/µL • RNA: 40 ng-cm/µL
Concentrations determined by absorbance measurements with NanoDrop Spectrophotometers are reported in terms of mass units (i.e., ng/µL).	**Tip:** Unit conversion calculators are available on the internet for the conversion of concentrations from mass to molar units based upon the specific sequence of the sample.

Pathlength & concentration

NanoDrop instrument pedestal measurements utilize pathlengths of 1.0 mm to 0.03 mm (model dependent). The pathlengths are the distance between the optical fibers embedded in the upper and lower pedestals. As described in the previous section, the pathlength is one component of Beer's Law and must be taken into account when calculating sample concentrations using absorbance measurements.

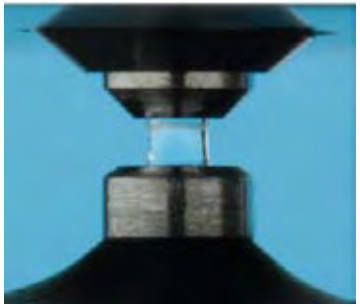
As the pathlength gets shorter, the ability to measure higher concentrations without saturating the internal detector increases. **Although the upper detection limit of the internal spectrometer is ~ 1.5 Absorbance units, the NanoDrop sample retention technology allows for the use of shorter pathlengths, thereby extending the absorbance range of the instrument.**

The graphic below illustrates how utilizing pathlengths less than the standard 10 mm (1 cm) cuvette pathlength enables higher concentrations of samples to be measured without making sample dilutions.



10 mm pathlength max
concentration = 75 ng/µL dsDNA

10 mm pathlength max
Abs value = 1.5



1 mm pathlength max
concentration = 750 ng/µL dsDNA

Equivalent to max Abs value
of 15 when normalized to a
10 mm pathlength

0.2 mm pathlength max
concentration = 3,750 ng/µL dsDNA

Equivalent to max Abs value of 75
when normalized to a
10 mm pathlength

Model	Detection limits for dsDNA
Thermo Scientific NanoDrop One/One^{C} Spectrophotometer	0.2* to 27,500 ng/µL
Thermo Scientific NanoDrop Eight Spectrophotometer	2.0 to 10,000 ng/µL
Thermo Scientific NanoDrop Lite Plus Spectrophotometer	2.0 to 1,500 ng/µL

* Denotes lower detection limit when using 10 mm path cuvette.

Purity ratios

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., real-time PCR).

260/280 ratio

- A 260/280 ratio of ~ 1.8 is generally accepted as "pure" for DNA.
- A 260/280 ratio of ~ 2.0 is generally accepted as "pure" for RNA.
- The reported 260/280 ratio is dependent on the pH and ionic strength of the buffer used to make the blank measurement and sample measurements. Acidic solutions will under-represent the 260/280 ratio by 0.2 – 0.3, while a basic solution will over-represent the ratio by 0.2 – 0.3.*

** William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: BioTechniques 22:474-481 (March 1997)*

Tip: If the ratio is appreciably different than the accepted target described above, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 260 nm.

Tip: It is important to ensure that the blank and sample buffers are at the same pH and ionic strength.

260/230 ratio

- The 260/230 values for a "pure" nucleic acid are often higher than the respective 260/280 values and are commonly in the range of 1.8 – 2.2.

Tip: If the ratio is appreciably different than the accepted target, it may indicate the presence of residual phenol, guanidine, magnetic beads, carbohydrates or proteins.

Tip: It is important to ensure that the blank and sample buffers are at the same pH and ionic strength. A low 260/230 ratio may indicate an issue with the buffer used for the blank measurement. Refer to the Troubleshooting section on page 16 for more information.

Performance Verification

All NanoDrop Spectrophotometers include a diagnostic application which allows the user to run a Performance Verification procedure to confirm that the instrument is working within specifications.

Wavelength verification (automatic)	• Wavelength verification 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 PV-1 in conjunction with the Performance Verification 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.
Performance Verification Solution	• PV-1 is a standard manufactured exclusively for use with NanoDrop Spectrophotometers and is available from Thermo Fisher Scientific and its distributors. • The PV-8 Calibration Kit (used for the NanoDrop Eight Spectrophotometer performance verification procedure) includes 2 PV-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 Performance Verification Solution.

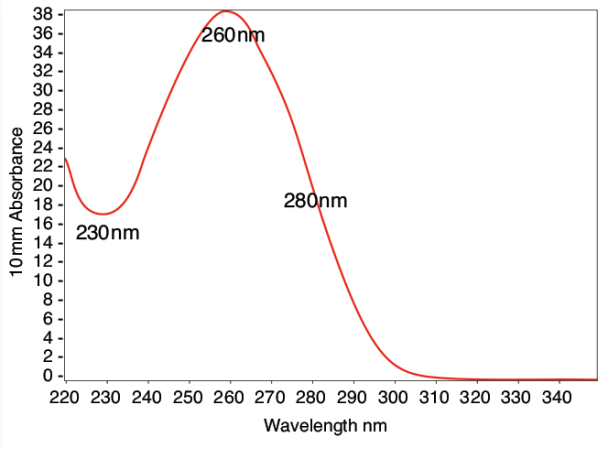
Standard vs control

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 PV-1 Performance Verification solution is the only acceptable standard for use with NanoDrop Spectrophotometers.
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.	• In general, most DNA ladders (without added dyes) are appropriate for use as routine laboratory control solutions to monitor the reproducibility between replicates and monitor 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.

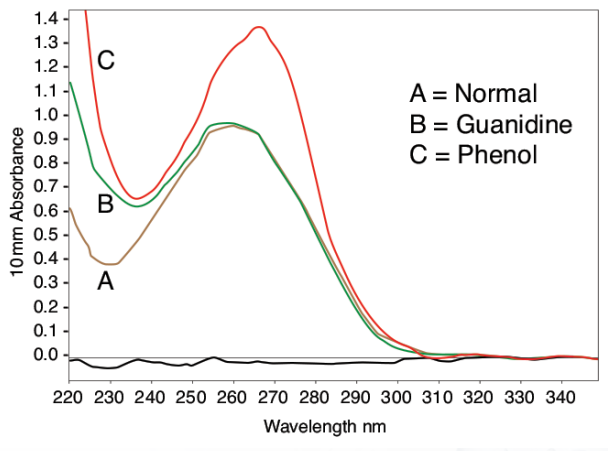
Tip: Ensure all controls are stored as recommend by the manufacturer. Do not use controls past the stated expiration date.

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

A typical nucleic acid sample will have a very characteristic profile as shown in the figure below, left. In the figure below, right, the shifts in the peaks and troughs of samples B and C, compared to sample A, illustrate how contaminants can affect the spectra of nucleic acid samples.



Typical nucleic acid sample spectrum



Comparison of nucleic acid sample spectra with and without 2 common contaminants

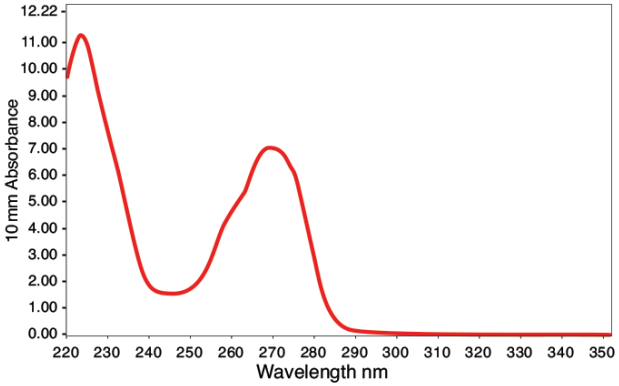
Purity ratios

Although 260/280 or 260/230 ratios are commonly used indicators to assess sample quality, ratios may sometimes fall within the range typically associated with “pure” samples but wavelength shifts in the trough (from 230 nm to 235 nm) or the sample peak (from 260 nm to 270 nm) may identify the sample to be of poor quality. Listed below are three common sources of contaminants:

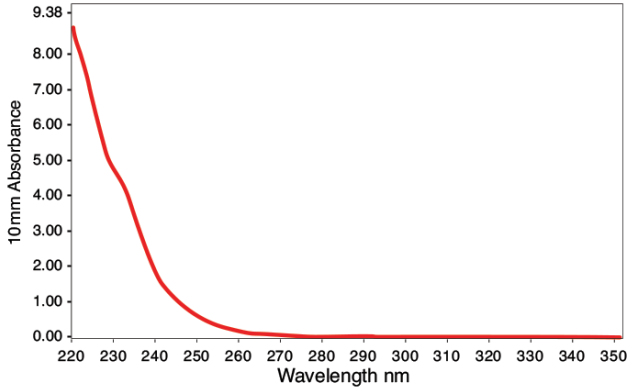
- Phenol/Trizol extraction – residual reagent contamination may be indicated by abnormal spectra between 220 to 240 nm as well as by shifts in the 260 to 280 nm region.
- Column extraction – residual guanidine may contribute to a peak near 230 nm and a shift in the trough from 230 nm to ~ 240 nm.
- Magnetic Beads – residual beads may cause light scatter and result in abnormal spectra.
- Samples with concentrations approaching the lower limit of 2 ng/µL may result in unacceptable 260/280 and/or 260/230 ratios.

Common reagents

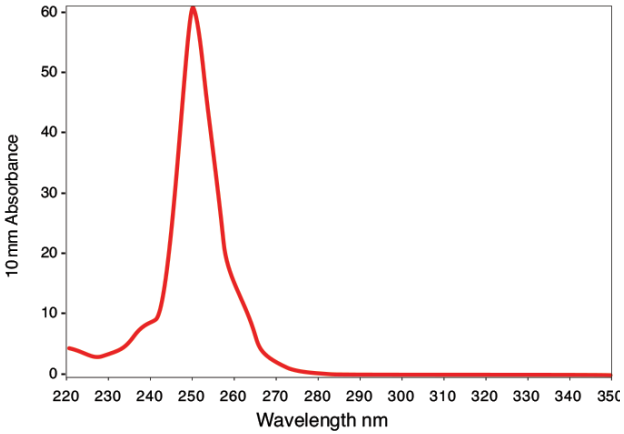
Below are several examples of reagents commonly used with nucleic acids that have absorbance in the 220 – 240 nm range. Note: Phenol also exhibits significant absorbance between 260 – 270 nm which may shift the peak and result in an overestimation of the nucleic acid concentration.



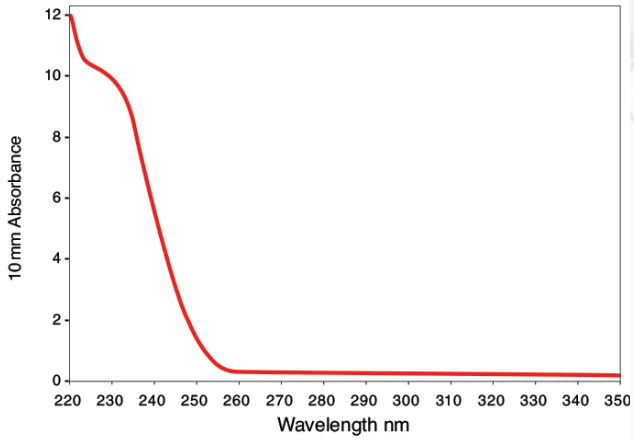
Phenol



EDTA



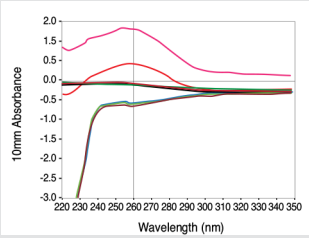
Guanidine Isothiocyanate



Guanidine HCl

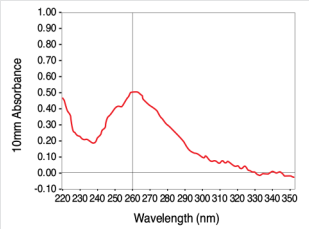
Tip: For samples with ratios that fall outside the expected optimal values, it is suggested that the user refer to the manufacturer of the isolation kit for additional information regarding protocol optimization and troubleshooting.

Unusual spectra



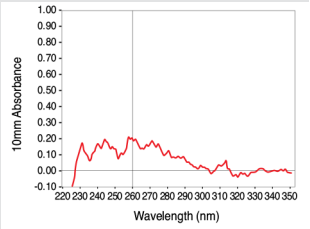
Negative values associated with some spectra indicate that either the pedestals were very dirty when the blank measurement was made or that a sample was used to make a blank or reblank measurement.

Suggestion:
Clean pedestal and measure new blank.



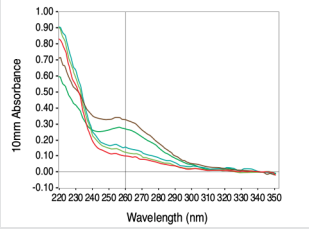
A ragged appearance throughout a spectrum may indicate a bad blank.

Suggestion:
Clean pedestal and measure new blank.



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

Suggestions:
Clean and recondition both measurement surfaces, then measure a new blank.
Increase sample volume to ensure proper column formation.



A very high 230 nm absorbance value relative to the sample is indicative of contaminants such as carbohydrates, peptides, phenols, urea, humic acid or guanidine isothiocyanate in the sample. It may also be the result of using an improper solution when making the blank measurement.

Suggestions:
Empirically determine if downstream applications are affected by samples that exhibit this type of profile.

Contact isolation kit manufacturer for additional information about optimizing the isolation protocol.

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 solution is homogeneous by heating and/or gentle vortexing, as appropriate.
Dirty pedestal	• Clean and recondition the pedestal surfaces prior to the start of the measurement session. • Follow the suggestions in the **Blank Measurements** section on page 9, prior to making sample measurements.
Multiple measurements	• Use fresh aliquots for each pedestal measurement. **Tip:** Multiple measurements of the same aliquot may 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.

Reproducibility

Concentrations not within expected range

- Ensure samples fall within the linear detection range of the instrument.

> **Tip:** Refer to the table of model-specific detection limits on page 12 for guidance.

- Ensure sample solution is homogeneous by gentle vortexing, as appropriate.
- Confirm that the blank solution and sample solvent are the same material.
- Clean and recondition the pedestal surfaces prior to the start of the measurement session.
- Ensure the appropriate application is selected as concentration calculations utilize constants specific to each sample type.

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 5.

- 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 performance verification. If the instrument is out of calibration, 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 5. • Run the Intensity Check diagnostic. Refer to the model-specific user guide for additional information.

Q: Do nucleic acids require purification prior to measurement on NanoDrop Spectrophotometers?

A: Yes. As with any spectrophotometer, absorbance measurements are not specific for a particular nucleic acid and will be affected by the presence of nucleotides and other molecular contaminants which absorb at 260 nm.

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

A: Although 1 µL volumes are usually sufficient for most pedestal-based sample measurements, increasing the sample size to 2 µL will ensure proper column formation for samples with reduced surface tension properties.

Q: What are the nucleic acid detection limits?

A: The detection limits vary according to the NanoDrop model as shown below:

Model	Detection limits for dsDNA
Thermo Scientific NanoDrop One/One^{C} Spectrophotometer	0.2* to 27,500 ng/µL
Thermo Scientific NanoDrop Eight Spectrophotometer	2.0 to 10,000 ng/µL
Thermo Scientific NanoDrop Lite Plus Spectrophotometer	2.0 to 1,500 ng/µL

* Denotes lower detection limit when using 10 mm path cuvette.

Q: Can I quantify other types of samples (e.g., proteins or microbial cell cultures) with NanoDrop Spectrophotometers?

A: Yes. The NanoDrop One/One^{C} and Eight Spectrophotometers can be used for protein A280 measurements, colorimetric assays, microbial cell cultures, as well as other UV-Vis measurements.

Q: Can I quantify nucleic acids labeled with fluorescent dyes?

A: Yes. The Microarray application enables the simultaneous measurement of both the absorbance of nucleic acid and the absorbance of the fluorescent dye, allowing detection at dye concentrations as low as 0.12 picomole per microliter.

Q: Can I measure the emission of fluorescent dyes?

A: No. A fluorospectrometer is used to detect the emission of fluorescent dyes often used with nucleic acid samples.

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

A: Typically within 3% at the 1 mm pathlength.

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

A: Typically ± 2 ng/µL (standard deviation) for samples < 100 ng/µL and ± 2% (%CV) for samples > 100 ng/µL.

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 laboratory 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 on a dirty pedestal or using a solution with more absorbance than the sample of interest. 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 PV-1 Performance Verification solution should be used with the Performance Verification diagnostic in the instrument software. PV-1 is a proprietary potassium nitrate / nicotinic acid reference solution for testing the accuracy of NanoDrop One/One^{C}, Eight, and Lite Plus spectrophotometers.

Q: How do I calibrate NanoDrop Spectrophotometers?

A: The performance verification 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: Data is stored locally on the instrument (NanoDrop Lite Plus and NanoDrop One/One^{C}) or on the PC software database (NanoDrop One/One^{C} and NanoDrop Eight PC software packages).

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 **thermofisher.com/nanodrop**.

Phone: 1.302.479.7707
E-mail: **nanodrop@thermofisher.com**
thermofisher.com/nanodrop

thermo scientific

ThermoFisher SCIENTIFIC

Notes

For technical support, please contact:
Toll-free in US and Canada: 1.877.724.7690
Phone: 1.302.479.7707 | e-mail: nanodrop@thermofisher.com

Learn more at **thermofisher.com/nanodrop**

thermo scientific

T104 08/24