Protein A280

Thermo Scientific NanoDrop Spectrophotometers

Thermo
SCIENTIFIC

Protein A280

Contents

Introduction   This booklet is meant to provide some basic protein measurement support information for direct A280 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.

The patented NanoDrop™ sample retention system employs surface tension to hold 0.5 to 2 µL samples in place between two optical fibers.

Separate booklets for nucleic acid and protein colorimetric 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
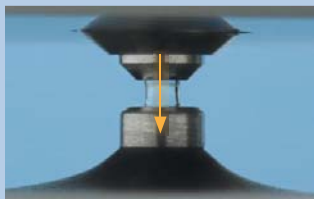
- Pipette 1 – 2 µL sample directly onto the measurement pedestal.

  Tip: 0.5 µL aliquots may be used for samples that have 10 mm equivalent absorbance values of 3.0 or higher (> 4.5 mg/mL BSA). NanoDrop 2000/2000c model only.

- 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.

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.

The Protein A280 method is applicable to purified proteins that contain Trp, Tyr residues or Cys-Cys disulphide bonds and exhibit absorbance at 280 nm. This method does not require generation of a standard curve and is ready for protein sample quantitation at software startup.

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.

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.
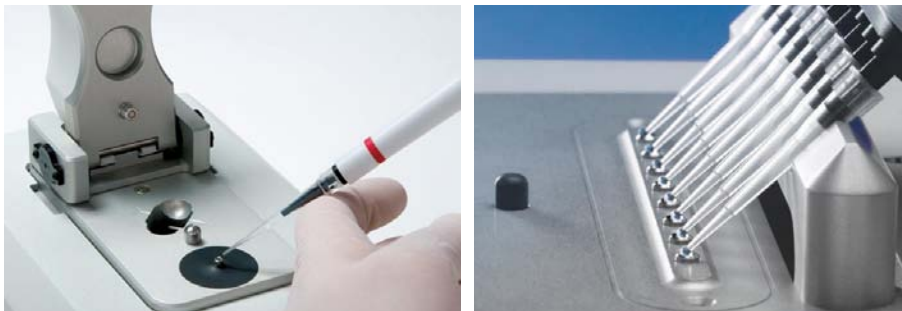
Below, recommended orientation for left- and right-handed sample dispensing on the NanoDrop 8000.
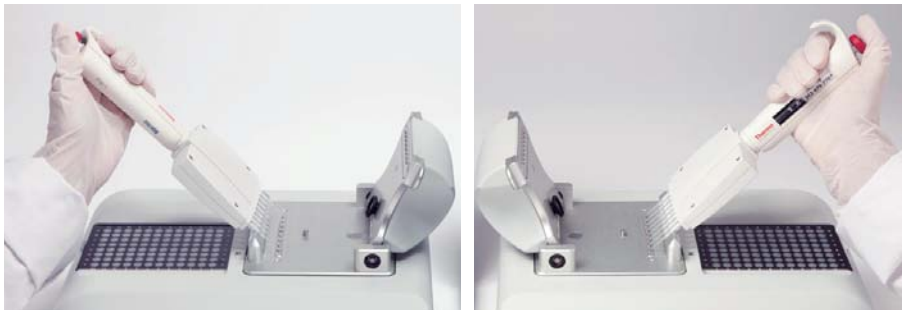
Cuvette Orientation
(NanoDrop 2000c only)

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 a 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 fully in solution or is not homogenous.

> Tip: It may be necessary to lightly vortex samples prior to sampling to ensure homogeneity.

Sample Preparation

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

Any molecule with absorbance at 280 nm will contribute to the total absorbance used to calculate sample concentrations. Triton X-100 and NP-9 are two examples of components found in common buffers that may contribute to the total absorbance values at 280 nm.

Pre-assessment of buffer compatibility with direct A280 measurements:

- Many buffers commonly used with protein samples contain components with significant absorbance in the UV region. Proteins in these types of buffers may not be suitable for direct quantitation using the A280 method.
- Proteins suspended in RIPA buffers may not be suitable for measurement using the A280 application. It is recommended that a colorimetric assay such as Pierce 660 nm be used for proteins suspended in RIPA buffers. Refer to the reagent manufacturer for more information regarding buffer compatibility.

Follow the steps below to determine if your buffer exhibits significant absorbance in the region of interest:

1. Perform the Cleaning and Reconditioning procedures outlined in the *Best Practices* section on page 6.

2. Open the A280 application. Load an aliquot of dH_2O onto the lower measurement pedestal and lower the sampling arm.

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

4. Pipette an aliquot of the sample buffer onto the pedestal, lower the arm and click **Measure**. The result should be a spectrum that varies no more than 0.04 absorbance (10 mm absorbance equivalent) from the baseline at 280 nm. If not, consider using a colorimetric method to quantitate the protein samples.
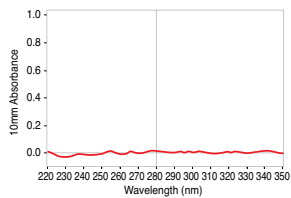
Example spectrum of buffer suitable for A280 protein quantitation
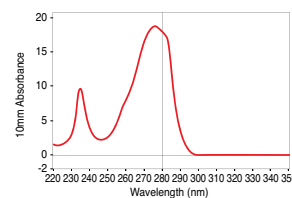
Example spectrum of buffer unsuitable for use with A280 protein quantitation

5. If the buffer is compatible with the A280 method, load a fresh aliquot of the buffer onto the lower measurement pedestal and lower the sampling arm. Click **Blank**.

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

> *Tip: 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.*

Measurements

Sample Measurements

1. After the blank measurement is complete, enter the sample name in the Sample ID box, and choose the appropriate sample type as described below:

Sample Type Option	Extinction Coefficient
BSA	6.67
IgG	13.7
Lysozyme	24.6
1 Abs = 1 mg/mL	Default general reference setting
E1%	User-entered mass extinction coefficient
E and MW	User-entered molar extinction coefficient and molecular weight

Refer to Sample Types Options in the *Calculations* section on pages 15 and 16 for more information.

2. Pipette an aliquot of the protein sample onto the lower measurement pedestal and lower the sampling arm. Click **Measure**.

> Tip: If measuring more than one sample at a time on the NanoDrop 8000, 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.
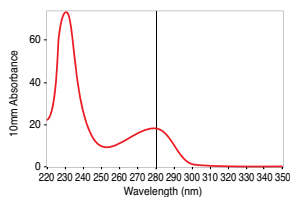
High concentration BSA sample
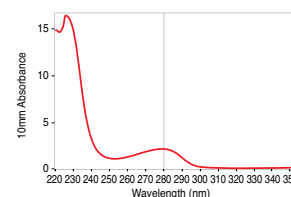
Low concentration BSA sample

> Tip: Refer to the Troubleshooting section on page 20 for more information.

Calculations

Absorptivity

The information in this section is a summary of the information presented in the Thermo Scientific Pierce products technical support bulletin # TR0006.2. Please refer to www.piercenet.com for additional information or to reach Pierce technical support.

Beer's Law states that molar absorptivity is constant (and the absorbance is proportional to concentration) for a given substance dissolved in a given solute and measured at a given wavelength.	$$A = \varepsilon * b * c$$ • A is the absorbance value (A) • ε is the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm • b is the path length in centimeters • c is the analyte concentration in moles/liter or molarity (M)
Solving the expression of Beer's Law for concentration yields the following equation:	$$c = A / \varepsilon \ b$$ • Dividing the measured absorbance of a protein solution by the molar extinction coefficient yields the molar concentration of the protein solution. Refer to the *Published Extinction Coefficients* section on page 13 for additional information regarding molar vs. mass concentration values.
The molar absorption coefficient of a peptide or protein is related to its tryptophan (W), tyrosin (Y) and cysteine (C) amino acid composition.	• At 280 nm, this value is approximated by the weighted sum of the 280 nm molar absorption coefficients of the three constituent amino acids, as described in the following equation: $$\varepsilon = (nW \times 5500) + (nY \times 1490) + (nC \times 125)$$ • n is the number of each residue • Stated values are the amino acid molar absorptivities at 280 nm

Calculations

Absorptivity

Absorptivity is wavelength specific for each protein and can be affected by buffer type, ionic strength and pH.

- The best extinction coefficient value is one that is determined empirically using a solution of the study protein at a known concentration.

  Tip: When determining extinction coefficients for a specific protein, it is important to use the same buffer as will be used for the general assay.

- Refer to the Pierce bulletin # TR0006.2 for additional information regarding the use of Pierce standards to calculate a "system-specific" extinction coefficient.

Protein sample concentrations are determined based on the absorbance at 280 nm, the extinction coefficients determined by the selected sample type and a baseline correction.

- The correction normalizes for any baseline offset attributable to light scattering artifacts. The default wavelength for the baseline normalization is 340 nm.

  Tip: The user may manually enter a different wavelength to be used for the baseline normalization when using the NanoDrop 2000/2000c.

Calculations

Published Extinction Coefficients

Absorption coefficients (i.e., extinction coefficients) for many proteins have been compiled from the literature and reported in various handbooks. These values provide sufficient accuracy for most routine laboratory applications that require an assessment of protein concentration. Most sources report extinction coefficients for proteins measured at or near a wavelength of 280 nm in phosphate or other physiologic buffer.

Published Values

- Some resources provide coefficient values for specific proteins as the wavelength-dependent molar absorptivity coefficient (ε or extinction coefficient) with units of $M^{-1}cm^{-1}$.

- Some resources provide coefficient values for specific proteins as 1% (= 1 g/100 mL) solutions measured in a 1 cm cuvette. These values can be understood as percent solution extinction coefficients ($\varepsilon 1\%$) having units of $(g/100 \text{ mL})^{-1}cm^{-1}$ instead of $M^{-1}cm^{-1}$.

- Still other sources provide protein absorbance values for 0.1% (i.e., 1 mg/mL) solutions, as this unit of measure is more convenient and common for protein work than percent solution.

  Tip: The variation in reporting style underscores the importance of carefully reading stated values to be sure that the unit of measure is understood and applied correctly. Additional information is available in bulletin # TR0006.2 at www.piercenet.com.

Calculations

Published Extinction Coefficients

Conversions

• The relationship between molar extinction coefficient (ε molar) and percent extinction coefficient ($\varepsilon1\%$) is as follows:

$$(\varepsilon_{molar})* 10 = (\varepsilon1\%) \times (\text{molecular weight of protein})$$

Example:
Assume you want to determine the $\varepsilon1\%$ for a protein whose molar extinction coefficient is 43,824 $M^{-1}cm^{-1}$ and a molecular weight of 66,400 daltons. To determine the $\varepsilon1\%$ for this protein, rearrange the above equation as follows:

$$\varepsilon1\% = (\varepsilon_{molar}*10) / (MW)$$

$$\varepsilon1\% = (43,824*10) / (66,400 \text{ daltons})$$

$$\varepsilon1\% = 6.6$$

• To report concentrations in terms of mg/mL, an adjustment factor of 10 is used when using percent solution extinction coefficients. To convert from g/100 mL to mg/mL.

$$(A / \varepsilon1\%) *10 = \text{concentration in mg/mL}$$

Example:
Assume you obtain an 280 nm absorbance reading of 5.8A for a protein sample relative to your reference. To determine the calculated concentration of your sample (mg/mL), refer to the equation below:

$$C_{mg/mL} = (A/ \varepsilon1\%) \times 10$$

$$C_{mg/mL} = (5.8/6.6) \times 10$$

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

Tip: The NanoDrop software automatically includes the factor of 10 when reporting protein concentrations. The information presented above is for explanation purposes only.

Calculations

Sample Type Options

There are six sample types (options) available for purified protein analysis and concentration measurement using the A280 application on the NanoDrop models specified at the beginning of this guide. A description of each sample type is given below:

Representative Sample Types

BSA Protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (i.e., 10 mg/mL) Bovine Serum Albumin solution.	• Assuming a MW = 66,400 daltons, the molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$.
IgG Protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (i.e., 10 mg/mL) IgG solution.	• Most mammalian antibodies (i.e., immunoglobulins) have protein extinction coefficients (percent) in the range of 12 to 15. • For a typical IgG with MW = 150,000 daltons, this value corresponds to a molar extinction coefficient (ε) equal to 210,000 $M^{-1}cm^{-1}$.
Lysozyme Protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (i.e., 10 mg/mL) Lysozyme solution.	• Molar extinction coefficient for egg white lysozyme ranges from 36,000 to 39,000 $M^{-1}cm^{-1}$.

Calculations

Sample Type Options

Other Sample Types

|	
1 Abs= 1 mg/ml A general reference setting based on a 0.1% (i.e., 1 mg/mL) protein solution producing an Absorbance at 280 nm of 1.0 A (where the pathlength is 10 mm or 1 cm).	• Assume ε1% = 10 if no extinction coefficient information exists for a protein or protein mixture of interest and a rough estimate of protein concentration is required for a solution that has no other interfering substances. • Most protein extinction coefficients (ε1%) range from 4.0 to 24.0. Although any given protein can vary significantly from ε1% = 10, the average for a mixture of many different proteins will likely be approximately 10. *Tip: This option is useful when measuring a protein solution for which no absorptivity information is available.*
Other protein (ε + MW) The relationship between molar extinction coefficient (ε molar) and percent extinction coefficient (ε percent) is as follows: (ε molar) 10 = (ε percent) × (molecular weight of protein).	• Although the label of the field where one enters the extinction coefficient differs between NanoDrop models, e.g., ε /1000 or ε (x1000), the value entered would be the same. For example, for a protein with a molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210 in the ε window for all model types. • Enter the molecular weight in kilodaltons in the MW (kDal) field. *Tip: Use this option when the molar extinction coefficient ($M^{-1}cm^{-1}$) and molecular weight are known.*
Other protein (ε1%)	• Enter the mass extinction coefficient (L $gm^{-1}cm^{-1}$) for a 10 mg/mL (ε1%) solution of the respective reference protein.

Calculations

Pathlength & Concentration

NanoDrop instrument pedestal measurements utilize pathlengths of 1.0 mm to 0.05 mm (model dependent).

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 cuvette pathlength enables higher concentrations of samples to be measured without making sample dilutions.
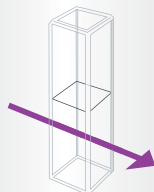
10 mm pathlength
max concentration
= ~ 2 mg/mL BSA
10 mm pathlength
max Abs value = 1.5

1 mm pathlength
max concentration
= ~ 20 mg/mL BSA
Equivalent to max Abs value of 15 when normalized to a 10 mm pathlength

0.2 mm pathlength
max concentration
= ~ 100 mg/mL BSA
Equivalent to max Abs value of 75 when normalized to a 10 mm pathlength

The table below lists the BSA detection limits** specific to each model.

Model	Absorbance Limits (10 mm equivalent)	Detection Limits for BSA**	Typical Reproducibility (minimum 96 replicates) (SD= mg/mL; CV= %)
NanoDrop 2000	0.03 to 300	0.1 mg/mL to 400 mg/mL	0.10 – 10 mg/mL: ± 0.10mg/mL >10 mg/mL: ± 2%
NanoDrop 2000c	0.003* to 300	0.010 mg/mL to 400 mg/mL	0.10 – 10 mg/mL: ± 0.10mg/mL >10 mg/mL: ± 2%
NanoDrop 8000	0.03 to 75	0.1 mg/mL to 100 mg/mL	0.15 – 5 mg/mL: ± 0.15 mg/mL >5 mg/mL: ± 2.5%
NanoDrop 1000	0.03 to 75	0.1 mg/mL to 100 mg/mL	0.10 – 10 mg/mL: ± 0.10 mg/mL >10mg/mL: ± 2%

* Denotes lower detection limit when using 10 mm path cuvette.
** The detection limits will vary based upon the extinction coefficients associated for specific proteins.

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.

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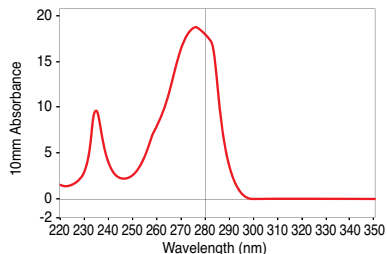
When troubleshooting sample measurements, it is important to utilize the sample spectrum as a primary guide.
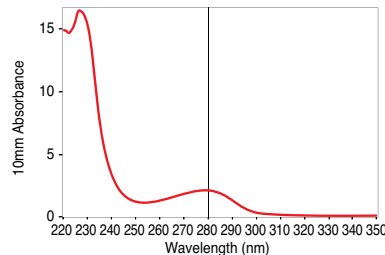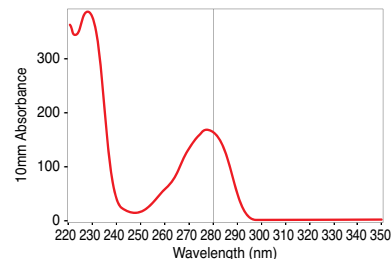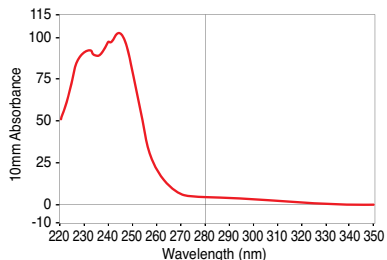

Typical Protein Spectrum at 280 nm (BSA in PBS)

Below are several examples of reagents commonly used with proteins with absorbance in the 220 – 350 nm range:
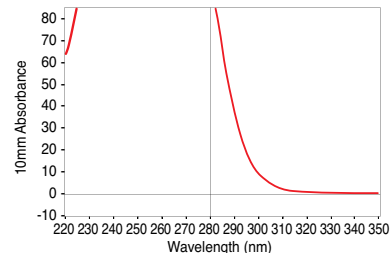

RIPA Buffer

Triton X-100

4M DTT

7M Urea and 2M Thiourea

Troubleshooting

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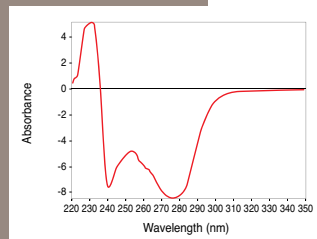
Negative values associated with some spectra indicate that either the pedestals were very dirty when the blank measurement was made or that a sample was used to make a blank or reblank measurement.

Suggestion:
Clean pedestal and measure new blank.
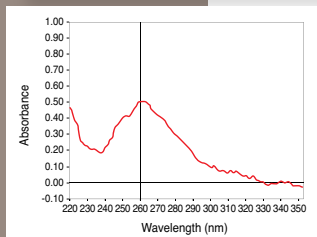
A ragged appearance throughout a spectrum may indicate a bad blank.

Suggestion:
Clean pedestal and measure new blank.
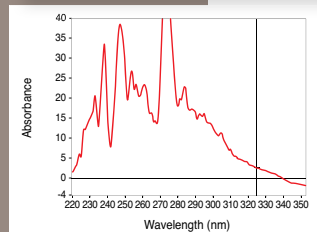
A jagged appearance throughout a spectrum may indicate a broken column.

Suggestions:
Clean and recondition both measurement surfaces, then measure new blank.

Increase sample volume to ensure proper column formation.
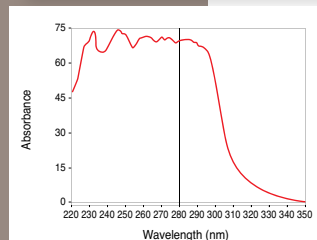
A jagged appearance at the top of the 280 mm peak most likely indicates detector saturation due to a highly concentrated sample.

Suggestion:
In cases like these, the data will be erroneous and should not be used for downstream work. Dilute the sample and remeasure.

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.

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.

Troubleshooting

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 17 for guidance.

- Ensure sample solution is homogeneous by gentle vortexing, as appropriate.

- Confirm that the reference (blank) solution and sample solvent are the same material.

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

- Ensure appropriate sample type 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 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: Can I quantify proteins using methods other than the A280 application on NanoDrop spectrophotometers?

A: Yes. Other options include purified proteins, a Proteins and Labels module for labeled antibodies and other protein incorporating fluorescent labels or intrinsic fluorescence. In addition, colorimetric assays such as the BCA, Bradford, Modified Lowry and Pierce 660 nm Protein assay are easy and quick to run. Custom methods may also be set up via the Method Editor on the NanoDrop 2000/2000c to analyze proteins, including peptides at 205 nm (available in the NanoDrop 2000/2000c operating software).

Q: I am using a colorimetric method (e.g., Bradford, BCA, etc.) to determine my protein concentration. Can I measure my sample using the A280 application on NanoDrop spectrophotometers?

A: Yes. The A280 application is most applicable to purified proteins. Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry are generally used for uncharacterized protein solutions and cell lysates. If you are using a colorimetric assay now, it is recommended that you continue to do so.

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: When is a 0.5 μL volume sufficient?

A: The small sample volume option is available when samples have 10 mm equivalent absorbance values of 3.0 or higher (> 4.5 mg/mL BSA).

Q: What are the protein detection limits?

A: The detection limits vary depending on the model and the protein type being measured. The table below presents the limits by model for Bovine Serum Albumin with a mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/mL) solution.

Model	Detection Limits for BSA
NanoDrop 2000	0.1 mg/ml to 400 mg/ml
NanoDrop 2000c	0.010* mg/mL to 400 mg/ml
NanoDrop 8000	0.1 mg/ml to 100 mg/ml
NanoDrop 1000	0.1 mg/ml to 100 mg/ml

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

Q: What if my samples are less concentrated than the above indicated lower detection limits?

A: Fluorescent dyes can be used in conjunction with the NanoDrop 3300 Fluorospectrometer to detect protein samples less concentrated than the limits indicated in the table on page 26. Refer to the NanoDrop 3300 Fluorospectrometer user guide found under the *Support* tab on www.nanodrop.com.

Q: What sort of accuracy should I expect with the NanoDrop 2000/2000c?

A: Typically within 2% at the 1 mm pathlength.

Q: What sort of reproducibility should I expect with the NanoDrop 2000/2000c?

A: Typically ± 0.1 mg/mL for samples < 10 mg/mL and ± 2% for samples > 10 mg/mL (BSA samples).

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

A: Typically within 2% at the 1 mm pathlength.

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.

Q: How do I calibrate NanoDrop spectrophotometers?

A: The Calibration Check diagnostic 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 save 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 version 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.
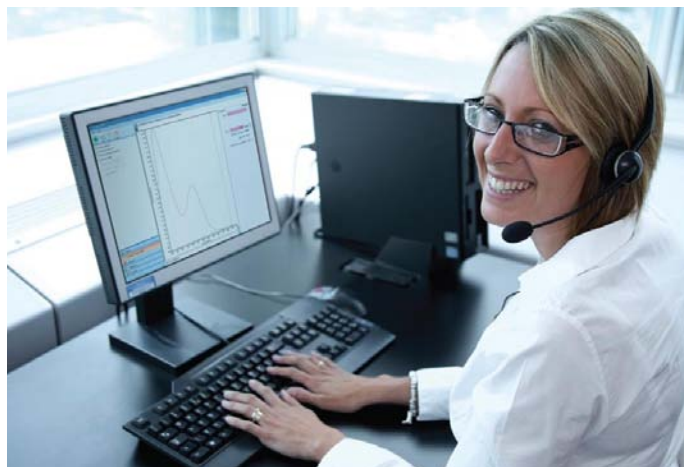
Additional technical information is available at: **www.nanodrop.com.**

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:

$$Absorbance = -\log \frac{intensity\ sample}{intensity\ blank}$$

RNA: 40 ng-cm/µL

ssDNA: 33 ng-cm/µL

(εmolar)* 10 = (ε1%) x (molecular weight of protein)

$$A = \varepsilon * b * c$$

dsDNA: 50 ng-cm/µL

0.2 mm pathlength

(A / ε1%) *10 = concentration in mg/mL

$$c = A / \varepsilon b$$

1 Abs = 1 mg/mL

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

Thermo Scientific NanoDrop Spectrophotometers
Protein A280

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

Thermo
SCIENTIFIC