thermoscientific

NanoDrop Micro-UV/Vis Spectrophotometer

NanoDrop Eight

User Guide

M020 NanoDrop Eight UG Revision Date April 2023

ThermoFisher SCIENTIFIC

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Contact your local distributor. For contact information go to:

http://www.thermofisher.com/NanoDropDistributors

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

Contents

About the Spectrophotometer

NanoDrop Eight Spectrophotometer

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

The Thermo Scientific™ NanoDrop™ Eight is a UV-Visible spectrophotometer developed for micro-volume analysis of a wide variety of analytes. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions. The instrument accurately measures up to 8 individual 1 µl samples in one measurement cycle. The software allows the user to measure samples using either the full 8-channel mode or a convenient single sample mode.

The NanoDrop Eight software is installed on a local PC used to control the instrument and view data.

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

Features

TheNanoDrop Eight spectrophotometer features the patented micro-volume sample retention system.

USB-B port

Connect the NanoDrop Eight to your PC via USB to operate the instrument using the PC software.

Accessories

This section lists the accessories included for use with the NanoDrop Eight.

PR-1 Pedestal Reconditioning Kit

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

PV-8 Performance Verification Kit

Kit containing plastic consumables and liquid photometric standard used to check instrument performance. For more information, see Performance Verification.

Instrument Detection Limits

Pathlength (mm)	Upper Detection Limit (10 mm Equivalent Absorbance)
1.0	12.5
0.2	90
0.1	200

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Instrument Set up

Register Your Instrument

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

To register your instrument

From any PC that is connected to the Internet, use any web browser to navigate to www.thermofisher.com/nanodropsw.

On the website, locate the Register Your Instrument button, and follow the instructions to register the instrument.

Computer Requirements

Required Windows Operating System

- Windows 10 Enterprise or Professional, build 1607 or greater

Minimum hardware configuration

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

Recommended hardware configuration

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

Update Software

Quickly and easily download and install the latest NanoDrop Eight software and release notes from our website. Follow the steps to update or upgrade the NanoDrop Eight software on a personal computer (PC). An Internet connection is required to download software. Navigate to www.thermofisher.com/nanodropsw and click on the NanoDrop Eight software tab. Follow the Instrument Software Download instructions.

To install or update NanoDrop Eight software on a PC

1. Insert the USB flash drive containing the installer software into an available USB port on your PC, or open the installation folder downloaded from the internet.
2. Launch **Start.exe** and click the **Software** button. The software installer will run.

Technical Support

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

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Fax: 302 792 7155
E-mail: nanodrop@thermofisher.com
Website: www.thermofisher.com/nanodrop

For International Support, please contact:

Contact your local distributor. For contact information go to:

http://www.thermofisher.com/NanoDropDistributors

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

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

Application Measurement Ranges

Detection Limits for All Applications

Note Detection limits provided in the following tables are approximate and apply to micro-volume measurements only; they are based on the instrument's photometric absorbance range (10 mm equivalent) of 0.04–200 A.

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
dsDNA	2.0 ng/µL	10,000 ng/µL	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL
RNA	1.6 ng/µL	8,000 ng/µL	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL
DNA Microarray (ssDNA)	1.3 ng/µL	495 ng/µL	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
Purified BSA by Protein A280	0.06 mg/mL	300 mg/mL	±0.10 mg/mL (for 0.10–10 mg/mL samples); ±2% for samples >10 mg/mL
IgG by Protein A280	0.03 mg/mL	145 mg/mL	
Purified BSA by Proteins & Labels	0.06 mg/mL	19 mg/mL	±0.10 mg/mL for 0.10–10 mg/mL samples
Protein BCA	0.2 mg/mL (20:1 reagent/sample volume); 0.01 mg/mL (1:1 reagent/sample volume)	8.0 mg/mL	2% over entire range 0.01 mg/mL over entire range
Protein Lowry	0.2 mg/mL (pedestal)	4.0 mg/mL (pedestal)	2% over entire range
Protein Bradford	100 µg/mL (50:1 reagent/sample volume) 15 µg/mL (1:1 reagent/sample volume)	8000 µg/mL 100 µg/µL	±25 µg/mL for 100–500 µg/mL samples ±5% for 500–8000 µg/mL samples ±4 µg/mL for 15–50 µg/mL samples ±5% for 50–125 µg/mL samples
Protein Pierce 660	50 µg/mL (15:1 reagent/sample volume) 25 µg/mL (7.5:1 reagent/sample volume)	2000 µg/mL 1000 µg/mL	±3 µg/mL for 50–125 µg/mL samples ±2% for samples > 125 µg/mL ±3 µg/mL for 25–125 µg/mL samples ±2% for samples >125 µg/mL

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

Detection limits for pre-defined dyes

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

[a] Values are approximate

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

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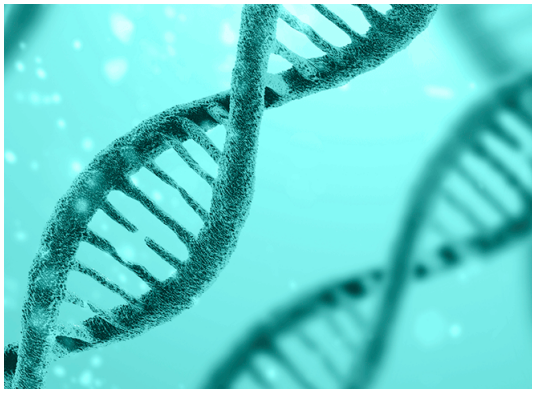
Nucleic Acid Applications

Measure dsDNA, ssDNA or RNA

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



Measure dsDNA, ssDNA or RNA

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

To measure dsDNA, ssDNA or RNA samples

NOTICE

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

Before you begin...

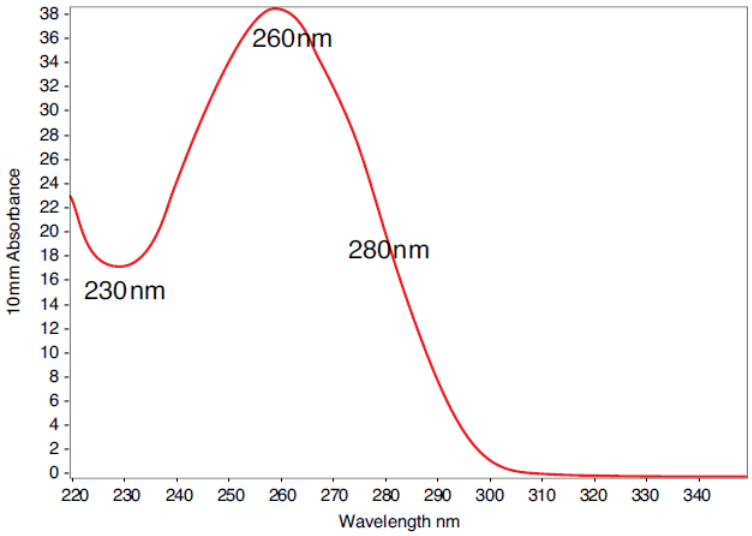
Before taking pedestal measurements with the NanoDrop Eight instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

To measure nucleic acid

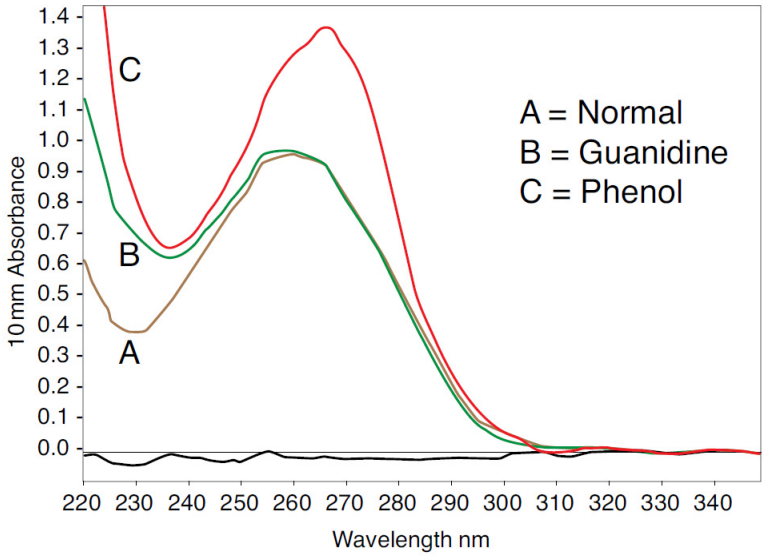
1. From the Home screen, select the **Nucleic Acids** tab, select your sample mode, and select **dsDNA, ssDNA or RNA**, depending on the samples to be measured.
2. Specify a baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select  **Blank** and wait for the measurement to complete.

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

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



Typical nucleic acid spectrum



Comparison of nucleic acid spectra with and without two common contaminants

Best practices for nucleic acid measurements

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

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

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

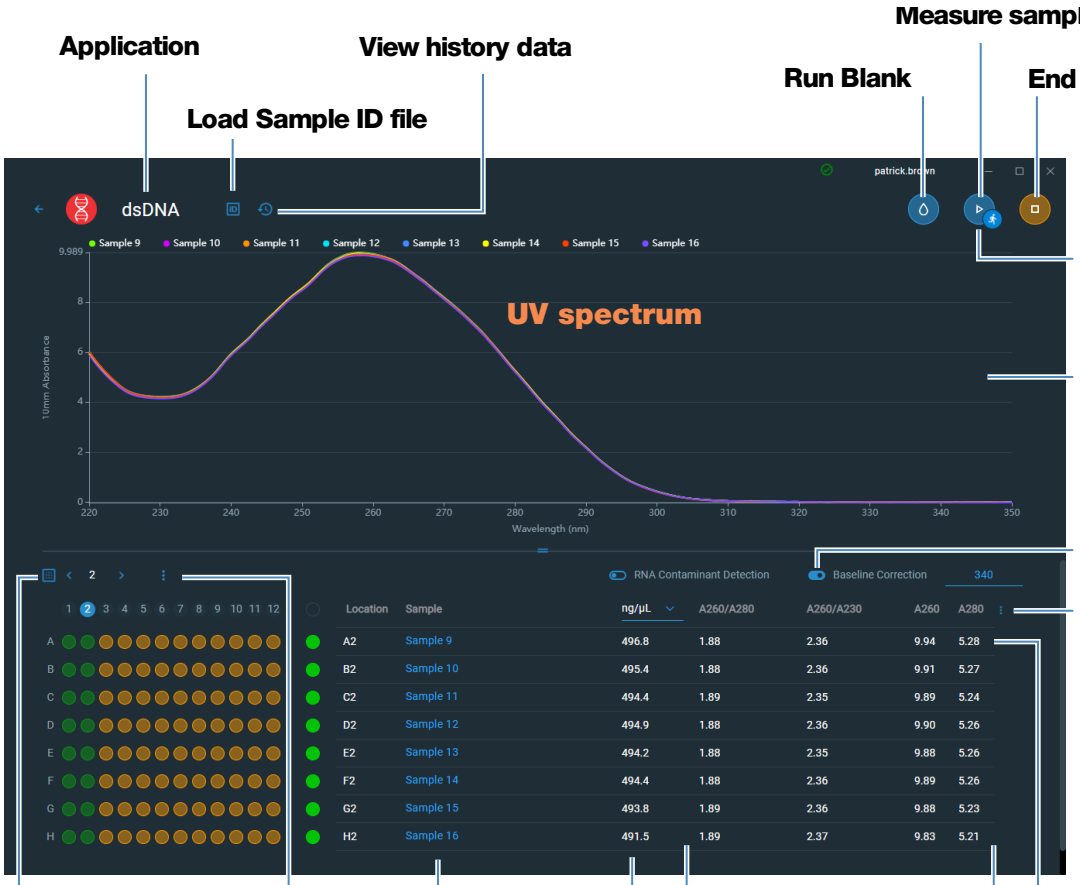
Related Topics

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

Nucleic Acid Reported Results

dsDNA measurement screen

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



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

dsDNA, ssDNA and RNA reported values

- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- Species
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction
- monitor wavelength
- pathlength used
- contaminant
- corrected

Settings for Nucleic Acid Measurements

Baseline Correction

For the dsDNA, ssDNA or RNA, enter baseline correction wavelength in nm or use default value (340 nm)

Optional user-defined baseline correction. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.

Calculations for Nucleic Acid Measurements

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

Extinction Coefficients vs Factors

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

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

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

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

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

or

$$c = A * f$$

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

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

Factors Used

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

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

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

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

Measured Values

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

A260 absorbance

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

A230 and A280 absorbance

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

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

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

- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration
- **Contaminant** - If a contaminant was identified by the Acclaro software, the contaminant will be displayed in this column.
- **Species** - The species of nucleic acid contaminant detected by the Acclaro sample intelligence technology
- **A260 absorbance**.
- **A280 absorbance**.
- **Baseline correction**
- **Monitor wavelength** - Enter an additional wavelength whose absorbance value you want included in the report.
- **Corrected** - Displays the corrected analyte concentration determined using the Acclaro software, if one is available.

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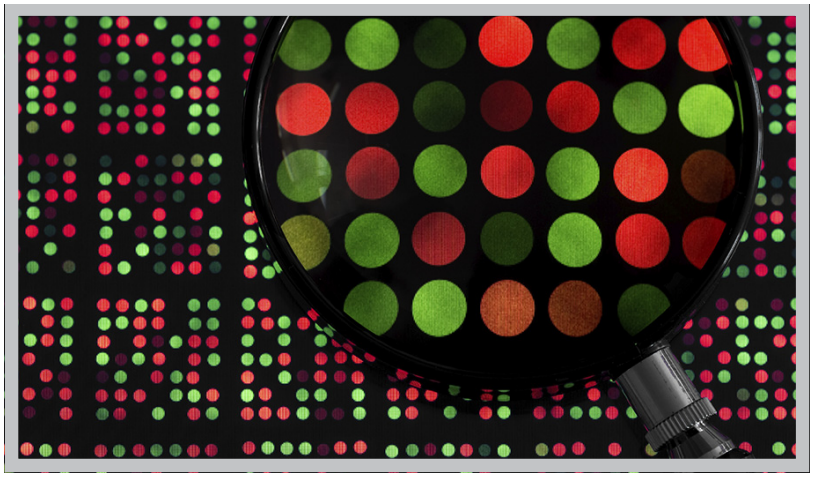
Measure Microarray

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



Measure Microarray Samples

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

To measure microarray samples

NOTICE

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

Before you begin...

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

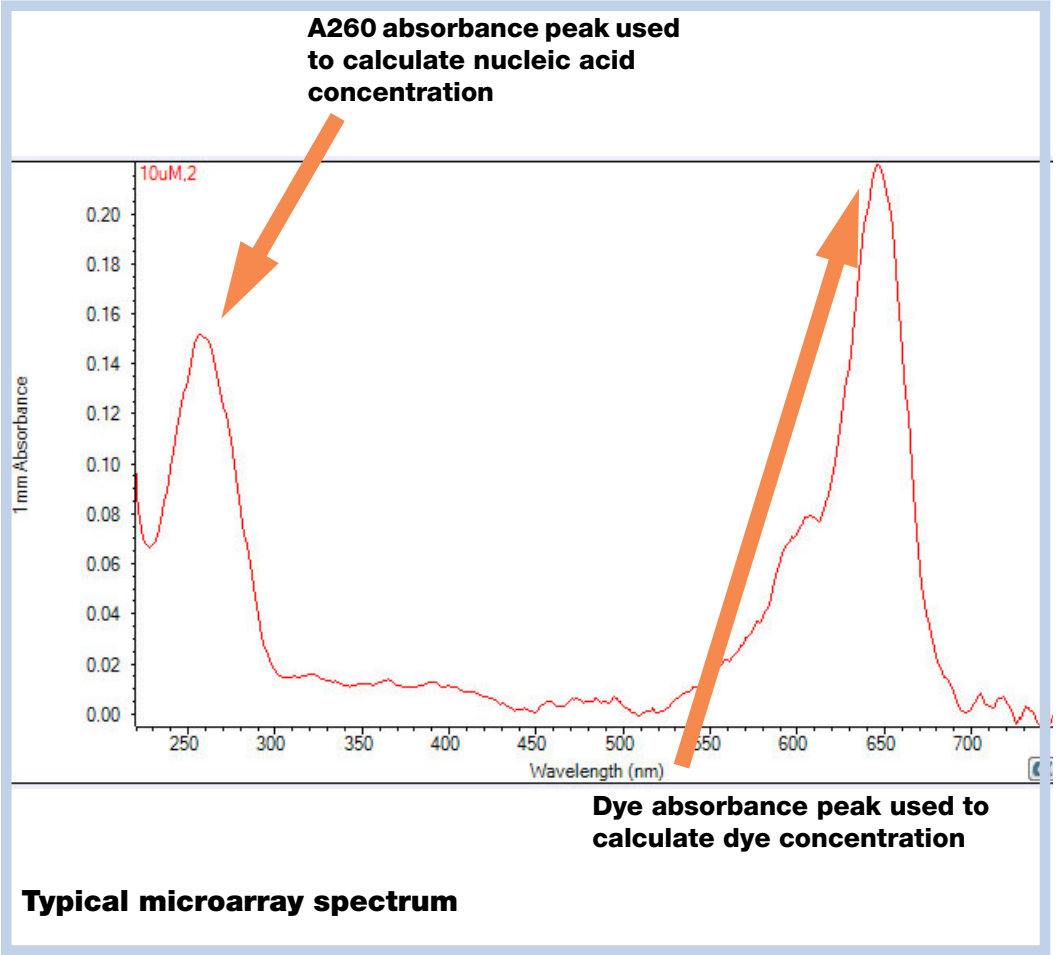
To measure a microarray sample

1. From the Home screen, select the **Nucleic Acids** tab and select **Microarray**.
2. Specify the sample type and factor and the type of dye(s) used.

   Tip: Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

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

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



Typical microarray spectrum

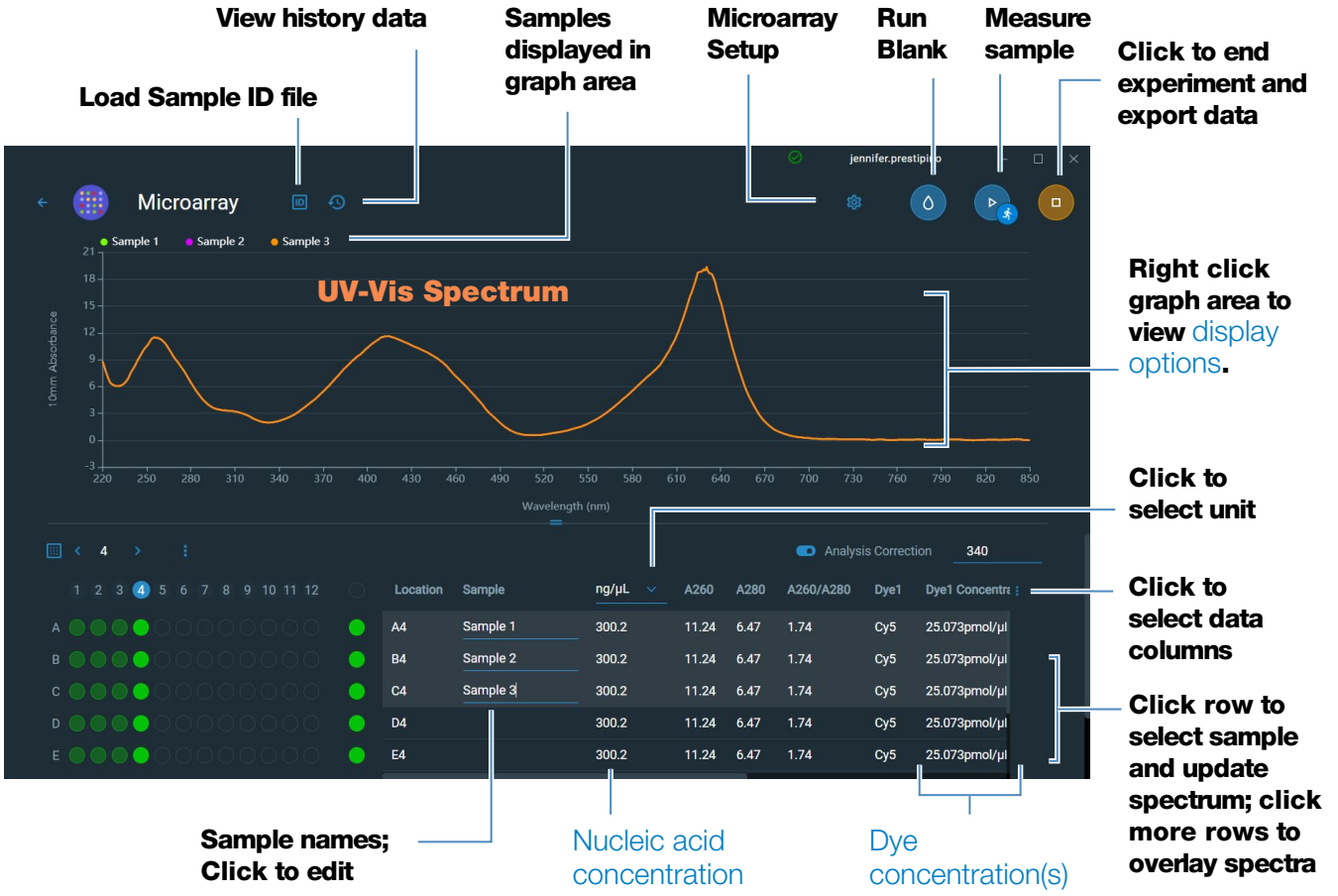
Related Topics

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

Microarray Reported Results

Microarray measurement screen

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



Note

- Analysis correction is performed at 340 nm (absorbance value at 340 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

Microarray reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:

- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260
- A260/A280
- dye 1/dye 2 concentration
- sample type
- analysis correction
- factor

Settings for Microarray Measurements

Microarray settings

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

Setting	Available Options	Description
Sample type and Factor	dsDNA (with non-editable factor of 50 ng-cm/µL)	Widely accepted value for double-stranded DNA
	ssDNA (with non-editable factor of 33 ng-cm/µL)	Widely accepted value for single-stranded DNA
	RNA (with non-editable factor of 40 ng-cm/µL)	Widely accepted value for RNA
	Oligo DNA with non-editable calculated factor in ng-cm/µL	Factor calculated from user-defined DNA base sequence. When selected, available DNA base units (i.e., G, A, T, C) appear as keys. Define sequence by selecting appropriate keys. Factor is calculated automatically based on widely accepted value for each base unit.

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

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

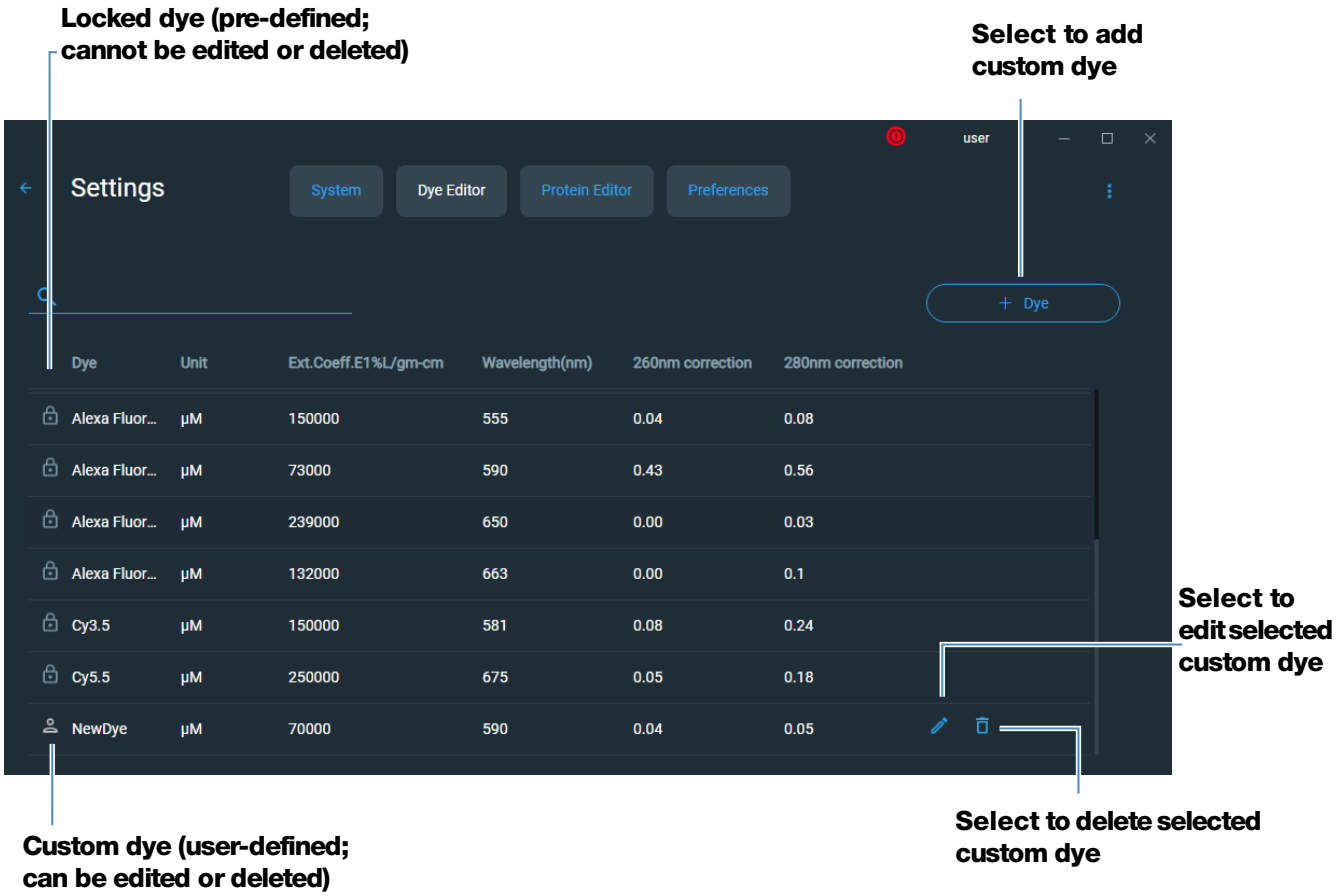
[b] The Analysis Correction affects the calculation for nucleic acid concentration only.

Dye/chromophore editor

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

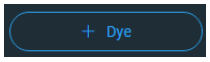
To access the Dye/Chromophore Editor, from the Home screen, select **Settings > Dye Editor**.

Dye Editor



These operations are available from the Dye/Chromophore Editor:

Add custom dye

- tap  to show Create Dye box
- enter unique **Name** for new dye
- select default **Unit** that will be used to display dye concentration
- enter dye's **Extinction Coefficient** (or molar absorptivity constant) in L/mole-cm (typically provided by dye manufacturer)
- specify **Wavelength** in nm (between 350 nm and 840 nm) that will be used to measure dye's absorbance
- specify dye's correction values at 260 nm and 280 nm
- select **Save**

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

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

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

Edit custom dye

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

- select custom dye
- select edit 
- edit any entries or settings
- click **Save**

Delete custom dye

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

- select custom dye
- click 

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

Calculations for Microarray Measurements

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

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

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

Available Options for Factors

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

Note: See Sample Type for more information.

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

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

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

Measured Values

A260 absorbance

Note: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 nm is zero in the displayed spectra. Also, for micro-volume absorbance measurements, the spectra are normalized to a 10 mm pathlength equivalent.

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

A280 absorbance

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

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

Dye absorbance

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

Dye correction

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

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

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

Related Topics

- Calculations for Nucleic Acid Measurements

Measure using a Custom Factor

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

Measure Nucleic Acid using a Custom Factor

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

To measure nucleic acid samples using a custom factor

NOTICE

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

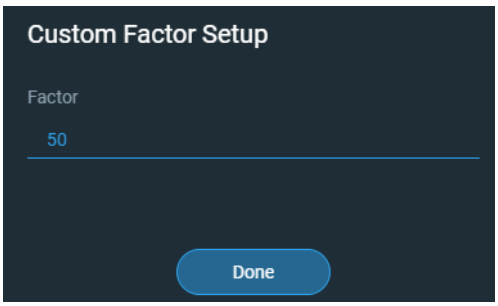
Before you begin...

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

To measure using a custom factor

1. From the Home screen, select the **Nucleic Acids** tab and select **Custom Factor**.

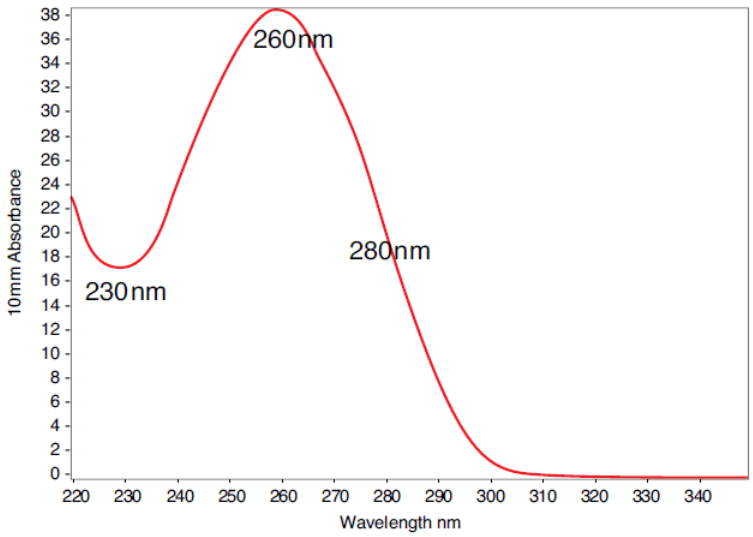
2. Enter the factor to be used for the calculations and specify a baseline correction if desired.



3. Pipette 1–2 µL blanking solution onto the lower pedestals and lower the arm.
4. Select  **Blank** and wait for the measurement to complete.

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

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



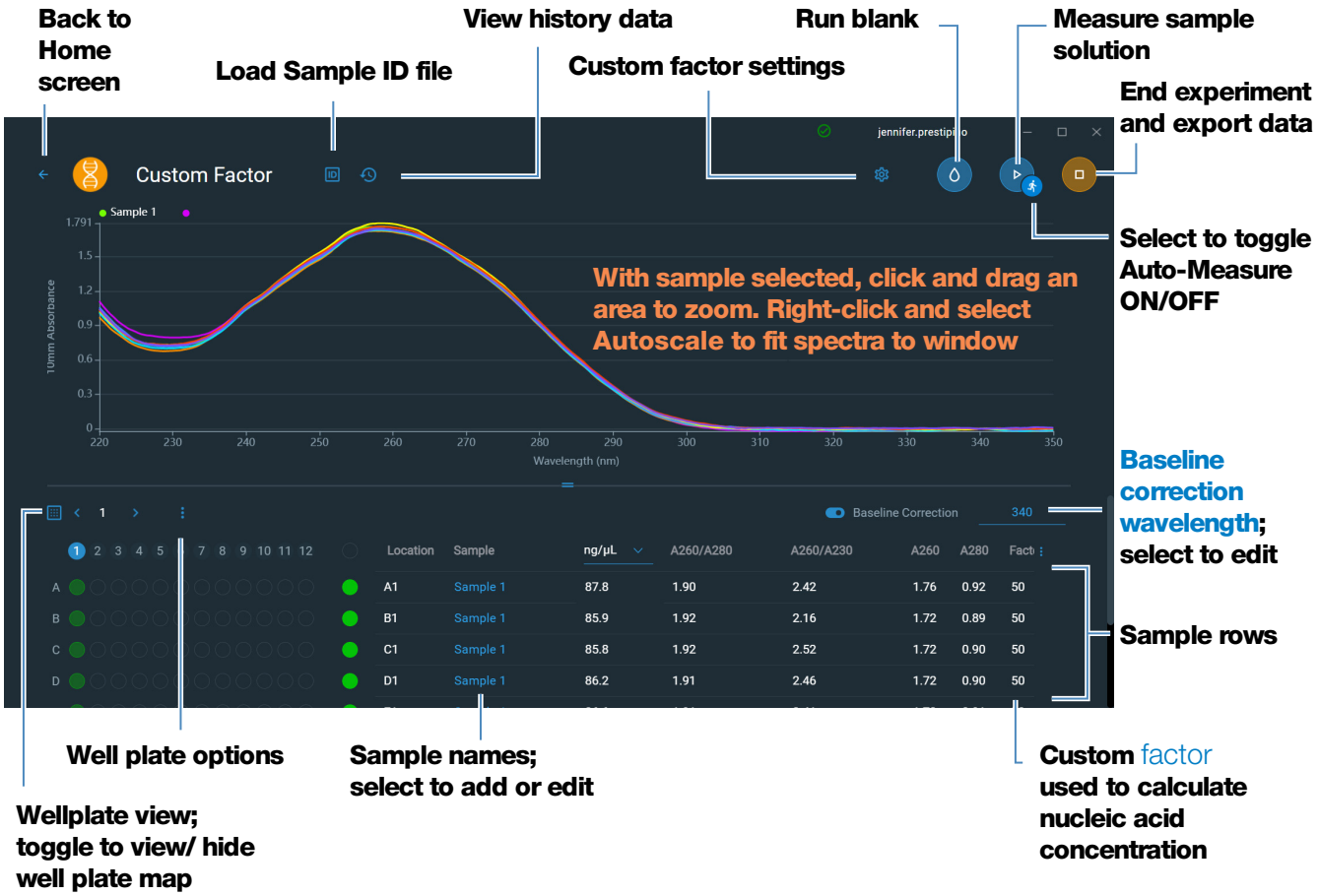
Typical nucleic acid spectrum

Related Topics

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

Custom Factor Reported Results

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



Tips:
Click sample row to select sample and update spectrum
Shift-click multiple sample rows to overlay spectra
Click a sample and hover locations on spectra to view measurement values

Related Topics

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

Settings for Nucleic Acid Measurements using a Custom Factor

To show the Custom Factor settings, from the Custom Factor measurement screen, select the settings icon  to view the **Custom Factor Setup**.

Setting	Available Options	Description
Custom Factor	Enter an integer value between 15 ng-cm/µL and 150 ng-cm/µL	Constant used to calculate nucleic acid concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength: $\mathbf{f = 1/(\varepsilon_{260} * b)}$ where: **f**= factor ε = molar extinction coefficient at 260 nm in ng-cm/µL **b** = sample pathlength in cm (1 cm for nucleic acids measured with the NanoDrop Eight instruments)
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	**Optional user-defined baseline correction**. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. NOTE: Baseline correction is selected from the measurement screen of the instrument software and is not shown in the Custom Factor Setup.

Related Topics

- Instrument Settings

Detection Limits for Nucleic Acid Measurements using a Custom Factor

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

To calculate upper detection limits for nucleic acid samples

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

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

For example, for a sample measurement using an extinction coefficient of 31, the equation looks like this:

(200 AU * 31 ng-cm/µL) = 6,200 ng/µL

Related Topics

- Detection Limits for All Applications

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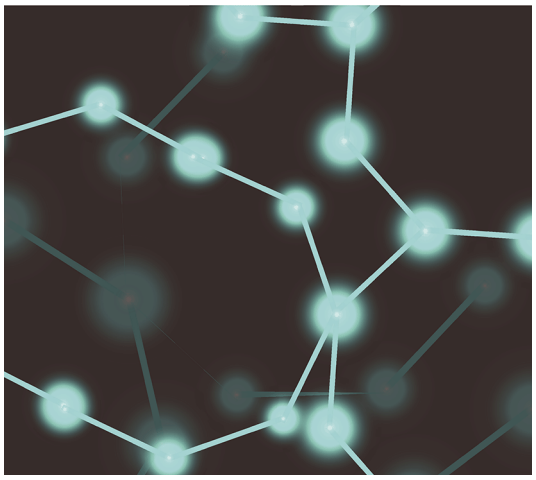
Measure Oligo DNA or Oligo RNA

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



Measure Oligo DNA or Oligo RNA

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

To measure Oligo DNA or Oligo RNA samples

NOTICE

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

Before you begin...

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

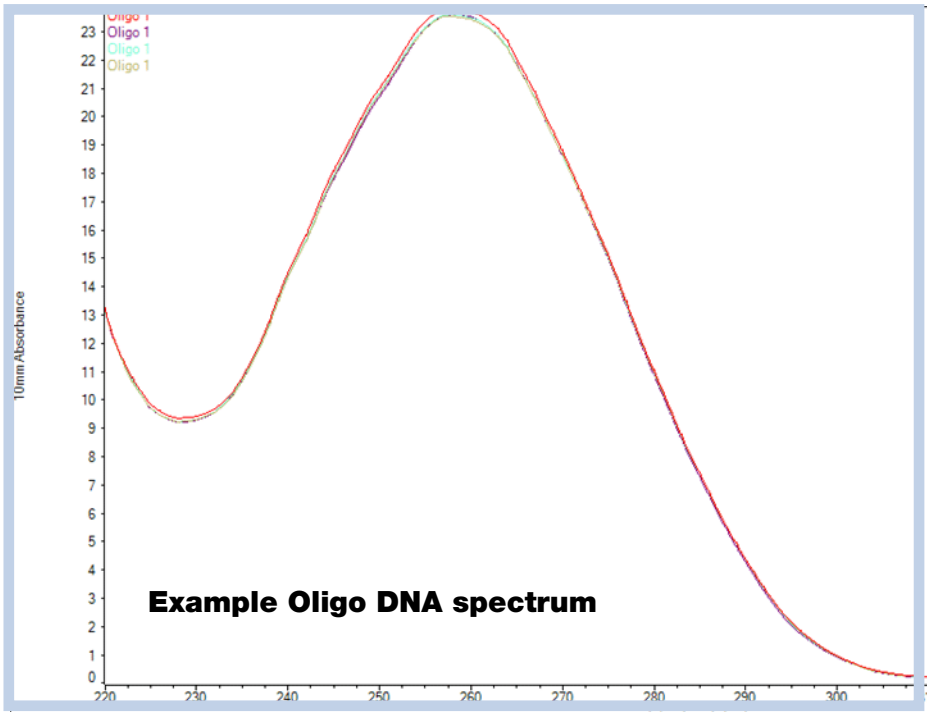
To measure an oligonucleotide sample

1. From the Home screen, select the **Nucleic Acids** tab and select either **Oligo DNA** or **Oligo RNA**, as needed.

2. Specify the Oligo base sequence for each individual channel and a baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select  **Blank** and wait for the measurement to complete.

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

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



Related Topics

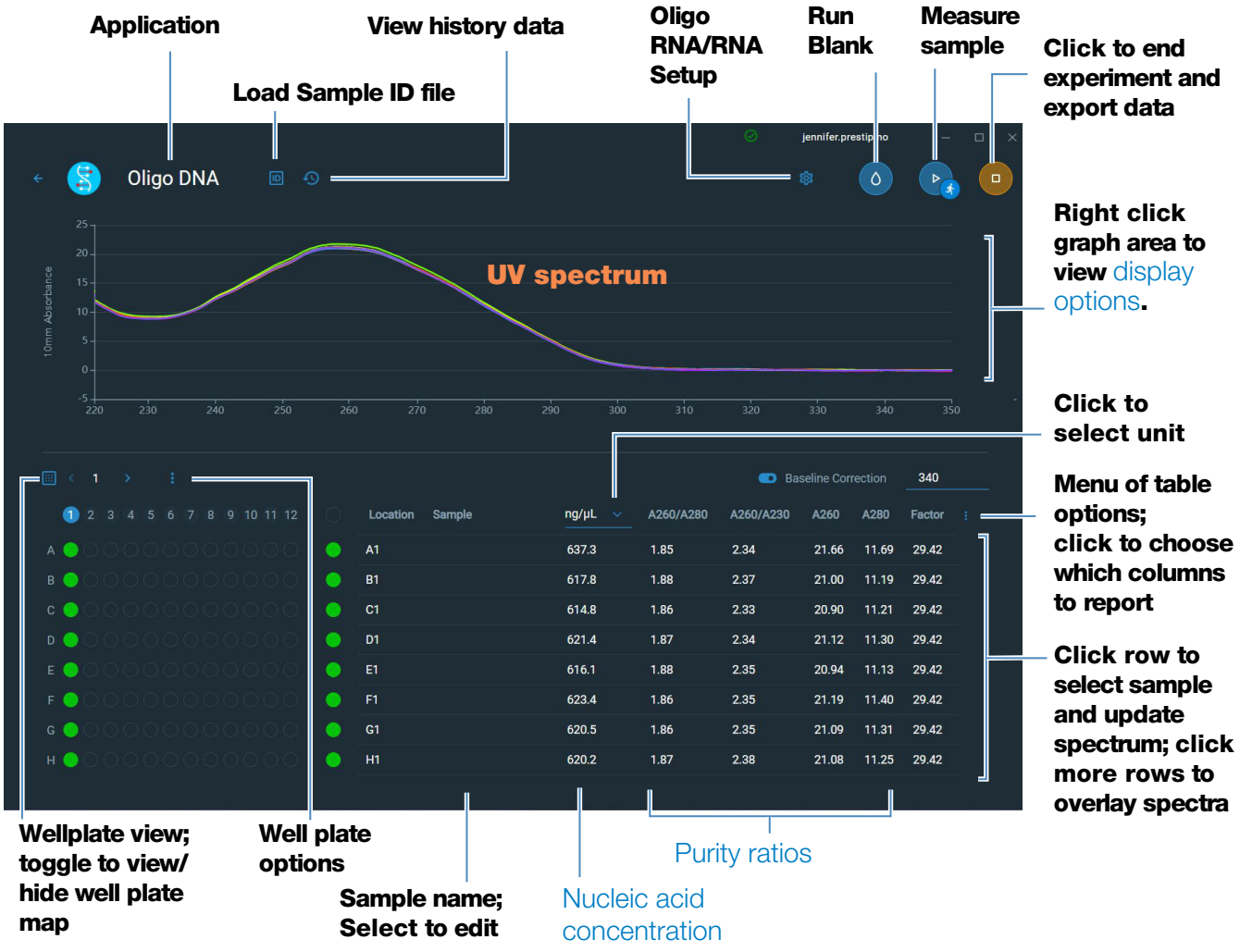
- Best Practices for Nucleic Acid Measurements

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

Oligo Reported Results

Oligo DNA and RNA measurement screen

For each measured sample, the Oligo DNA and Oligo RNA applications show the UV absorbance spectrum reported results. Here is an example:



Oligo DNA and Oligo RNA reported values

- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- oligo sequence
- baseline correction

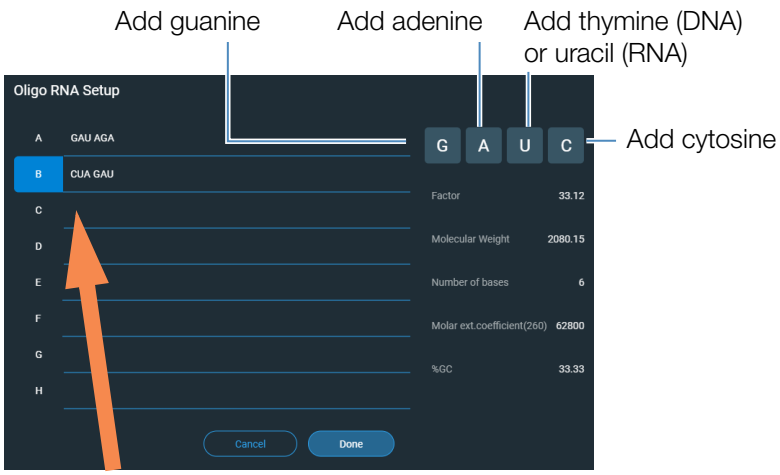
Note The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information.

Related Topics

- Basic Instrument Operations
- Oligo Calculations

Settings for Oligo DNA and Oligo RNA Measurements

The Oligo setup screen appears after you select the Oligo DNA or Oligo RNA application from the Nucleic Acids tab on the Home screen. From the Oligo DNA or RNA measurement screen, select the settings icon to view **Oligo DNA Setup** or **Oligo RNA Setup**.

Setting	Available Options	Description
Oligo Base Sequence	for DNA: Use the G, A, T and C keys to specify the DNA base sequence for RNA: Use the G, A, U and C keys to specify the RNA base sequence	Specify your DNA or RNA base sequences in each of the 8 channels. click the corresponding keys:  Base sequences specified in each channel You can also enter base sequence using the keyboard, or by copy and pasting a sequence from another application.
		Each time a base is added to the sequence, the software calculates the following: • **Factor**. Constant used to calculate oligonucleotide concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength: $f = 1/(\varepsilon_{260} * b)$ where: **f**= factor ε = molar extinction coefficient at 260 nm in ng-cm/µL **b** = sample pathlength in cm (1 cm for nucleic acids measured with the NanoDrop Eight instrument)

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

Related Topics

- Instrument Settings

Detection Limits for Oligo DNA and Oligo RNA Measurements

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

To calculate upper detection limits for nucleic acid samples

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

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

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

(200 AU * 55 ng-cm/µL) = 11,000 ng/µL

Calculations for Oligo DNA and Oligo RNA Measurements

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

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

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

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

Extinction Coefficients for Oligonucleotides

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

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

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

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

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

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

Measured Values

A260 absorbance

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

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

A230, A280 absorbance

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

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

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

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

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

Reported Values

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

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

Related Topics

- Calculations for Nucleic Acid Measurements

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Protein Applications

Measure Protein A280

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

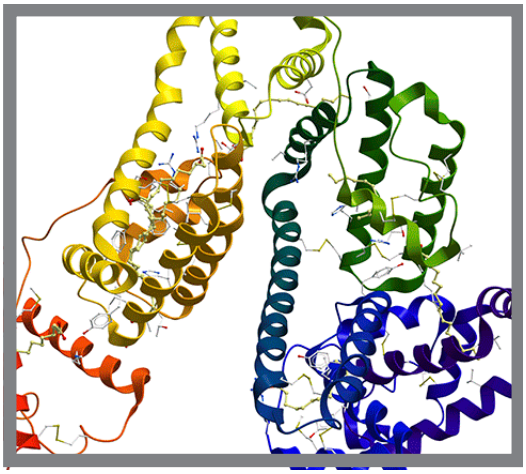
Measure A280 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A280

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

To measure Protein A280 samples

NOTICE

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

Before you begin...

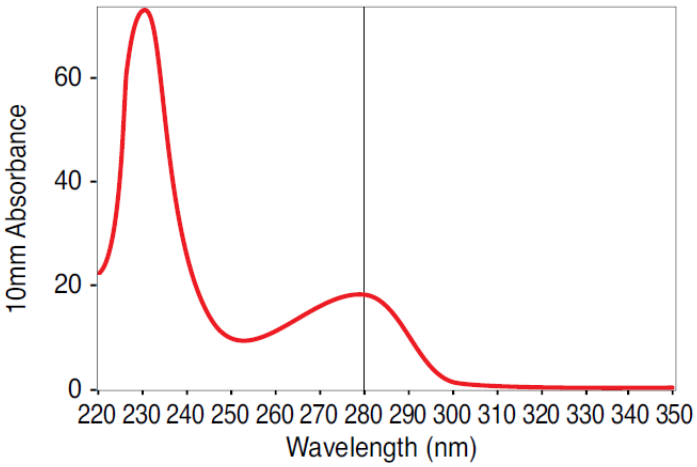
Before taking pedestal measurements with the NanoDrop Eight instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

To measure a Protein A280 sample

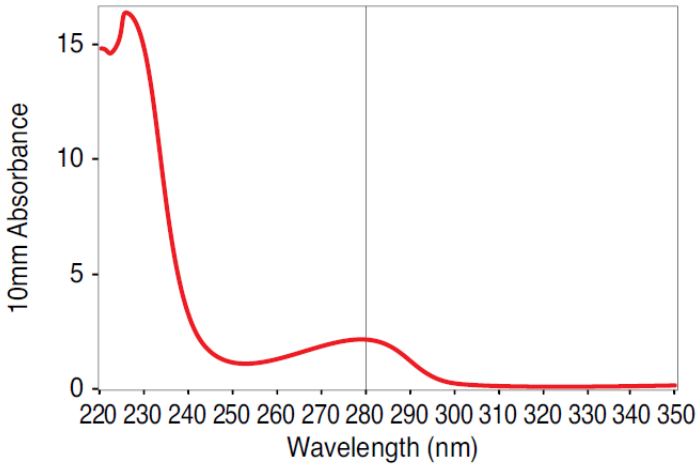
1. From the Home screen, select the **Proteins** tab and select **Protein A280**.
2. Specify a sample type and baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Click  **Blank** and wait for the measurement to complete.

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

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



High concentration BSA sample



Low concentration BSA sample

Best practices for protein measurements

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

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

- Ensure the sample absorbance is within the instrument's absorbance detection limits.

- Choosing a blank:
  - For the Protein A280 application, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 280 nm or 205 nm), you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.

  Note Buffers such as Triton X, RIPA, and NDSB contribute significant absorbance and are not compatible with direct A280 or A205 measurements.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned. (Proteins tend to stick to pedestal surfaces.)
  - Gently (but thoroughly) vortex samples before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 2 µL sample volume. See Recommended Sample Volumes for more information.

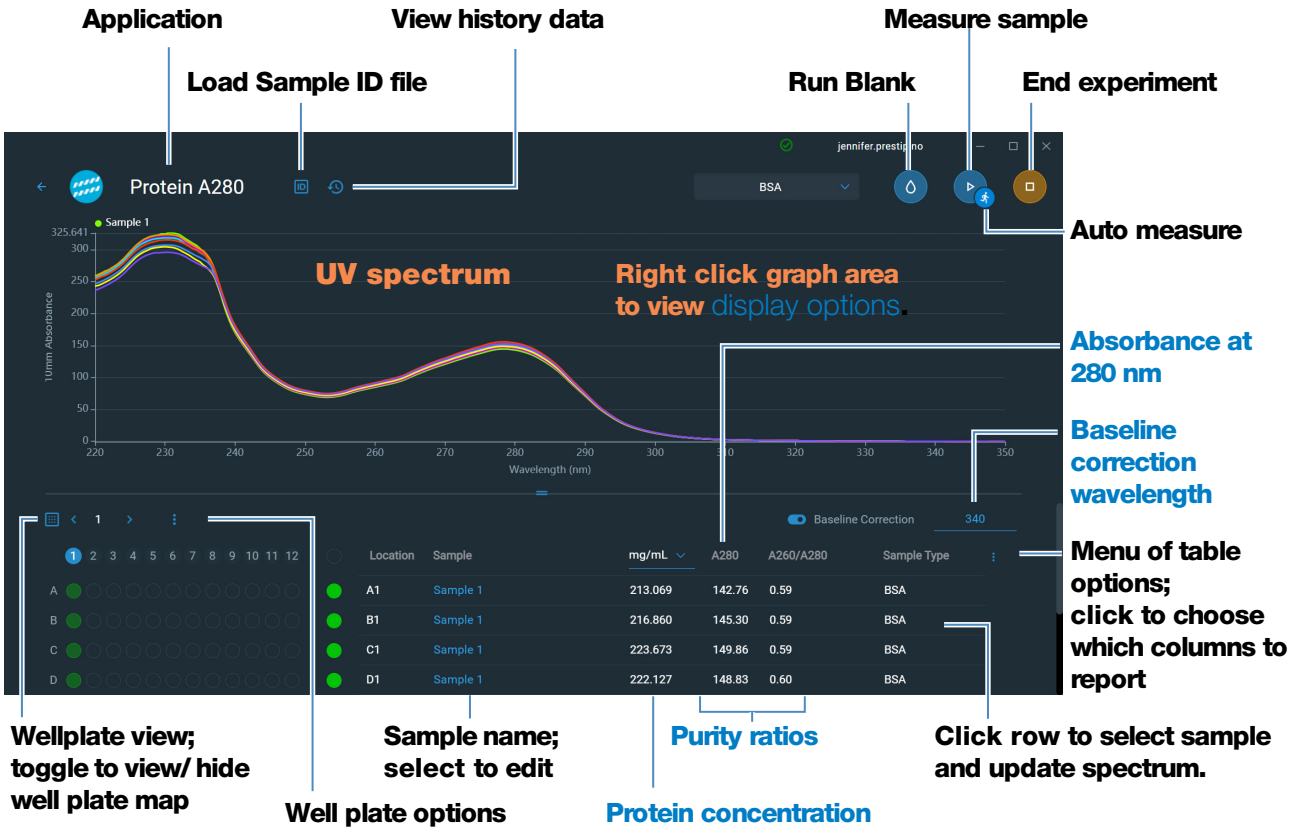
Related Topics

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

Protein A280 Reported Results

Protein A280 measurement screen

For each measured sample, this application shows the absorbance spectrum and a measurement results. Here is an example:



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

Protein A280 reported values

- Protein concentration
- Absorbance at 280 nm
- Purity ratio
- Sample type
- Baseline correction wavelength

- Baseline correction absorbance
- created on (date sample measurement was taken)
- monitor wavelength
- pathlength used
- Extinction Coefficient
- contaminant
- corrected

Related Topics

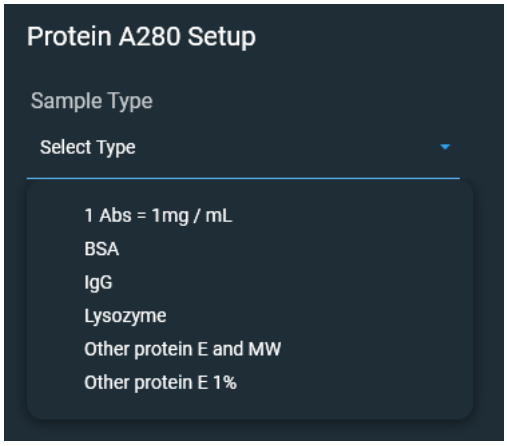
- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein A280 Measurements

To show the Protein A280 settings, from the Protein A280 measurement screen, select the settings icon  to view **Protein A280 Setup**.

Protein A280 settings

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



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

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

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type[a]	1 Abs = 1 mg/mL	General reference	Recommended when extinction coefficient is unknown and rough estimate of protein concentration is acceptable for a solution with no other interfering substances. Assumes 0.1% (1 mg/mL) protein solution produces 1.0A at 280 nm (where pathlength is 10 mm), i.e., $\varepsilon 1\% = 10$.
	BSA	6.7	Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient (ε) of 6.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) BSA solution. Assuming MW is 66,400 daltons (Da), molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$.
	IgG	13.7	Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (ε) of 13.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) IgG solution. Assuming MW is 150,000 Da, molar extinction coefficient at 280 nm for IgG is approximately 210,000 $M^{-1}cm^{-1}$.
	Lysozyme	26.4	Calculates lysozyme protein concentration using mass extinction coefficient (ε) of 26.4 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) lysozyme solution. Assumes molar extinction coefficient for egg white lysozyme ranges between 36,000 $M^{-1}cm^{-1}$ and 39,000 $M^{-1}cm^{-1}$.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
	Other protein (ε + MW)	User entered molar extinction coefficient and molecular weight	Assumes protein has known molar extinction coefficient (ε) and molecular weight (MW), where: $(\varepsilon_{molar})*10=(\varepsilon_{percent})*(MW_{protein})$ Enter MW in kiloDaltons (kDa) and molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (i.e., ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.
	Other protein (ε1%)	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (ε). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (ε1%) protein solution.

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

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	N/A	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. **Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction.

Protein editor

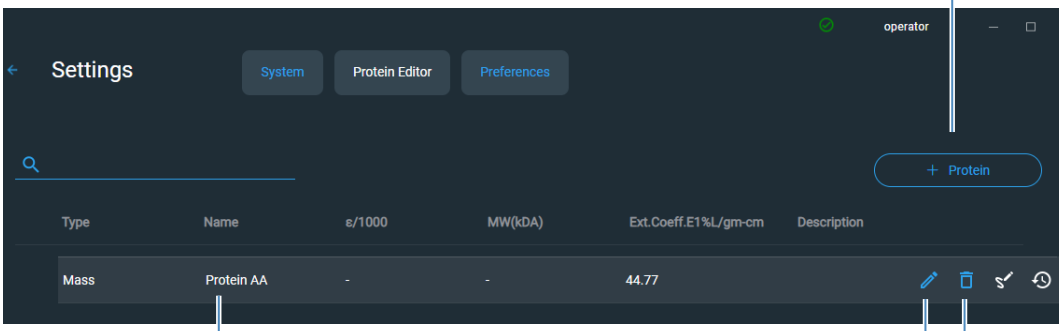
Use the Protein Editor to add a custom protein to the list of available protein sample types in Protein A280 Setup.

To access the Protein Editor:

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

Protein Editor

Click to add custom protein



Custom proteins (will appear in Sample Type list in Protein A280 Setup and Proteins & Labels Setup)

Click to edit selected custom protein

Click to delete selected custom protein

These operations are available from the Protein Editor:

Add custom protein

1. In Protein Editor, select + Protein to show the **New Protein Type** box.
2. Enter a unique **Name** for the new protein.
3. Enter a **Description** for the new protein.
4. Specify whether to enter **Molar Extinction** coefficient or **Mass Extinction** coefficient for custom protein.
   - If **Mass Extinction** coefficient is selected, enter mass extinction coefficient in L/gm-cm for 10 mg/mL (ε1%) protein solution.

– if **Molar Extinction** is selected,

– enter molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (that is, ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.

– Enter molecular weight (MW) in kiloDaltons (kDa)

5. Select **Save** to close the **New Protein Type** box.

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

Edit custom protein

1. In Protein Editor, click to select custom protein
2. Click  to show the **Edit Protein Type** box
3. Edit any entries or settings
4. Click **OK**

Delete custom protein

1. In Protein Editor, click to select a custom protein to delete
2. click 

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

Detection Limits for Protein A280 Measurements

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

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

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

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

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

(200 / 6.7) * 10 = 298.5 (or ~300)

Calculations for Protein A280 Measurements

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

Beer-Lambert Equation (solved for concentration)

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

where:

A = UV absorbance in absorbance units (AU)

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

b = pathlength in cm

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

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

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

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

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

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

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

Extinction Coefficients for Proteins

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

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

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

Available Options for Extinction Coefficient

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

Note: See Sample Type for details.

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

Published Extinction Coefficients

Published extinction coefficients for proteins may be reported as:

- wavelength-dependent molar absorptivity (or extinction) coefficient (ε) with units of $M^{-1}cm^{-1}$
- percent solution extinction coefficient (ε1%) with units of $(g/100\ mL)^{-1}cm^{-1}$ (i.e., 1% or 1 g/100 mL solution measured in a 1 cm cuvette)
- protein absorbance values for 0.1% (i.e., 1 mg/mL) solutions

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

The equation at the right shows the relationship between molar extinction coefficient (ε_{molar}) and percent extinction coefficient (ε1%).

Conversions Between ε_{molar} and ε1%

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

Example: To determine percent solution extinction coefficient (ε1%) for a protein that has a molar extinction coefficient of 43,824 $M^{-1}cm^{-1}$ and a molecular weight (MW) of 66,400 daltons (Da), rearrange and solve the above equation as follows:

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

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

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

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

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

Conversions Between g/100 mL and mg/mL

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

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

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

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

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

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

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

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

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

Measured Values

A280 absorbance

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

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

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

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

- **Contaminant** - Displays contaminant identified by the Acclaro software, if one is available.
- **Corrected** - Display the corrected analyte concentration determined using the Acclaro software, if one is available.

Measure Protein A205

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

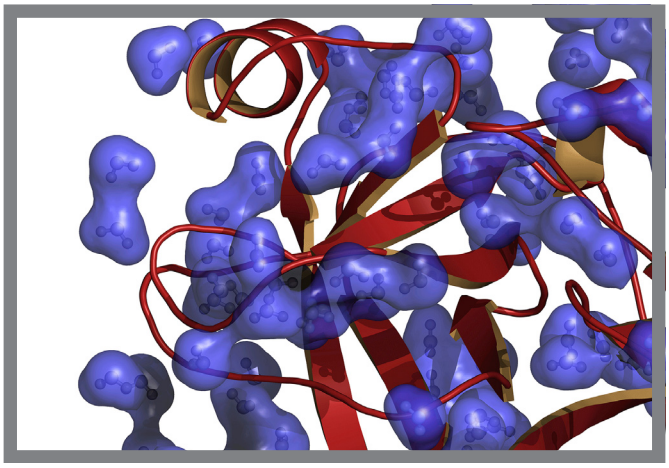
Measure A205 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A205

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

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

To measure Protein A205 samples

NOTICE

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

Before you begin...

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

To measure a Protein A205 sample

1. From the Home screen, select the **Proteins** tab and select **Protein A205**.
2. Specify a sample type and baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Click  **Blank** and wait for the measurement to complete.

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

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

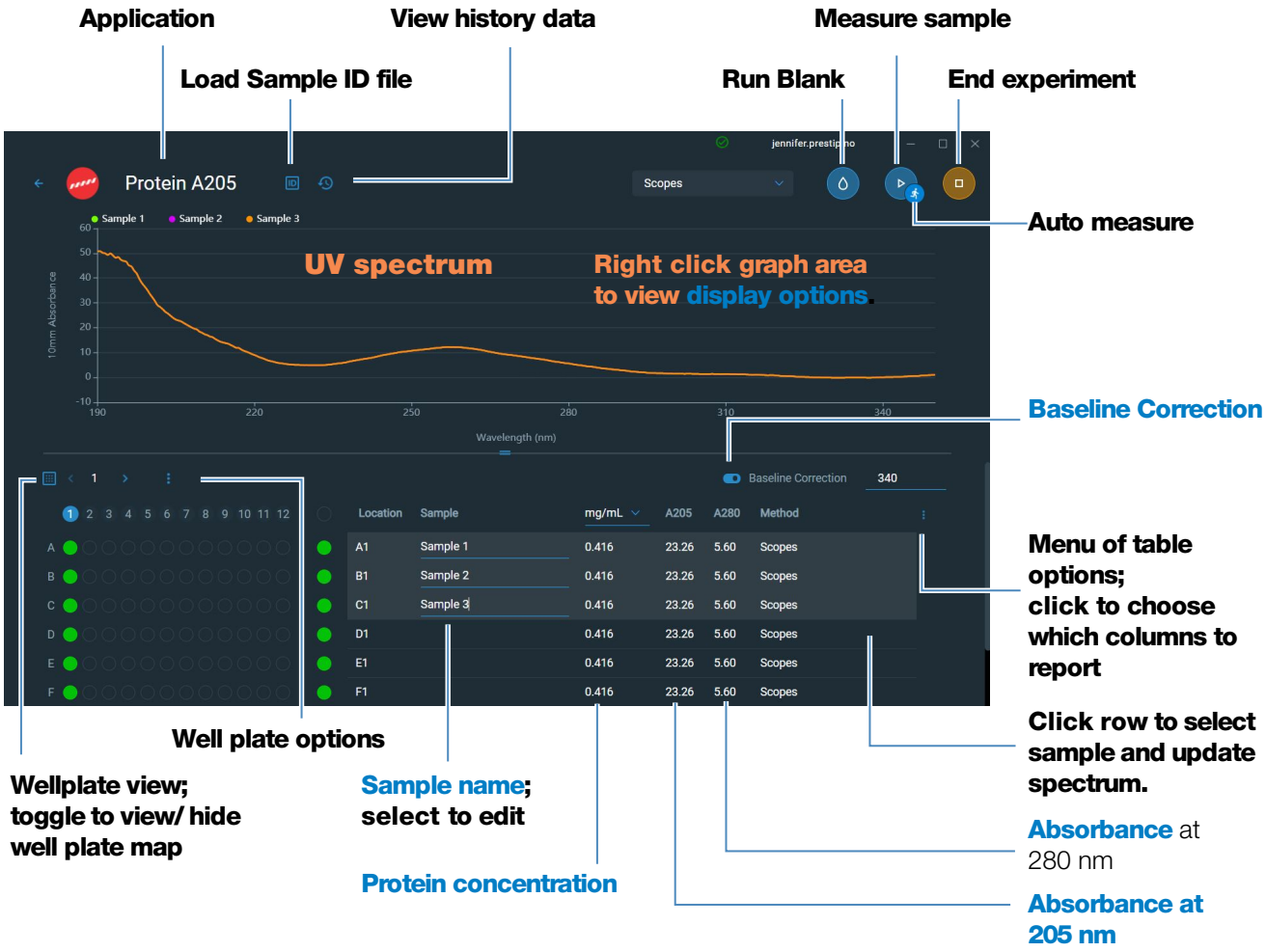
Related Topics

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

Protein A205 Reported Results

Protein A205 measurement screen

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



Protein A205 reported values

- Protein concentration
- Absorbance at 205 nm
- Absorbance at 280 nm
- Sample type
- Baseline correction

- created on (date sample measurement was taken)
- monitor wavelength
- pathlength used
- Extinction Coefficient

Related Topics

- Basic Instrument Operations
- Protein A205 Calculations

Settings for Protein A205 Measurements

To show the Protein A205 settings, from the Protein A205 measurement screen, select Protein A205 Setup  .

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type	31	31	Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 31
	Scopes	27 + 120 * (A280/A205)	Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
	Other protein (Ɛ1%)	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (Ɛ). Enter mass extinction coefficient in L/gm-cm for 1 mg/mL (Ɛ0.1%) protein solution.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	N/A	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength. **Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction.

Related Topics

- Instrument Settings

Calculations for Protein A205 Measurements

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

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

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

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

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

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

Available Options for Extinction Coefficient

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

Note: See Sample Type for details.

Measured Values

A205 absorbance

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

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

A280 absorbance

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Proteins and Labels

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

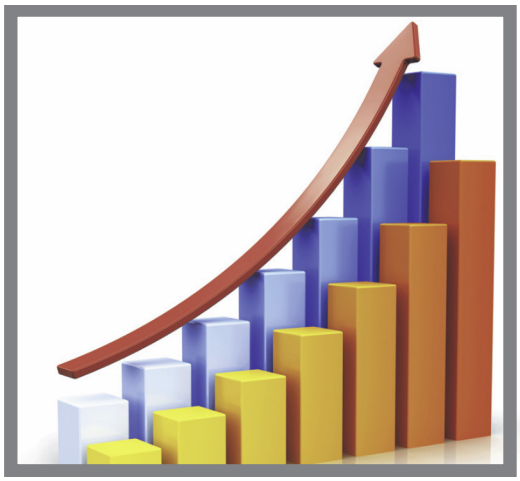
Measure Labeled Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Labeled Protein Samples

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

To measure labeled protein samples

NOTICE

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

Before you begin...

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

To measure a labeled protein sample

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

2. Specify the sample type and the type of dye(s) used.

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

3. Pipette 1–2 µL of the blanking solution onto the lower pedestal and lower the arm.

4. Tap **Blank** and wait for the measurement to complete.

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.

5. Lift the arm and clean all pedestals with a new laboratory wipe.

6. Pipette 2 µL sample solution onto the pedestal and lower the arm.

7. Start the sample measurement:

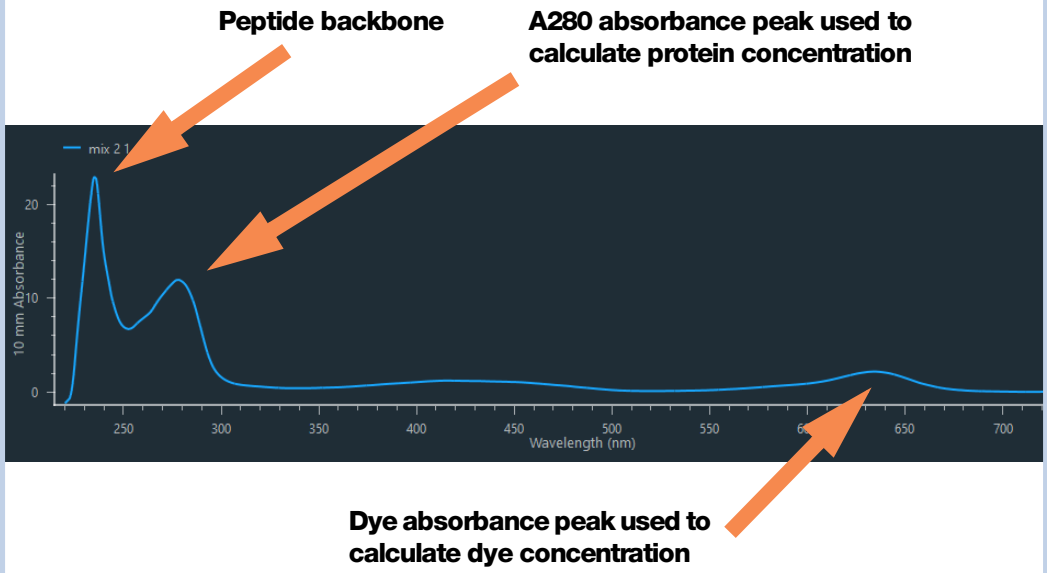
   – If Auto-Measure is On, lower arm;

   – if Auto-Measure is off, lower arm and select **Measure**.

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

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

9. Lift the arm and clean all pedestals with a new wipe.



Typical sample spectrum measured with Proteins & Labels

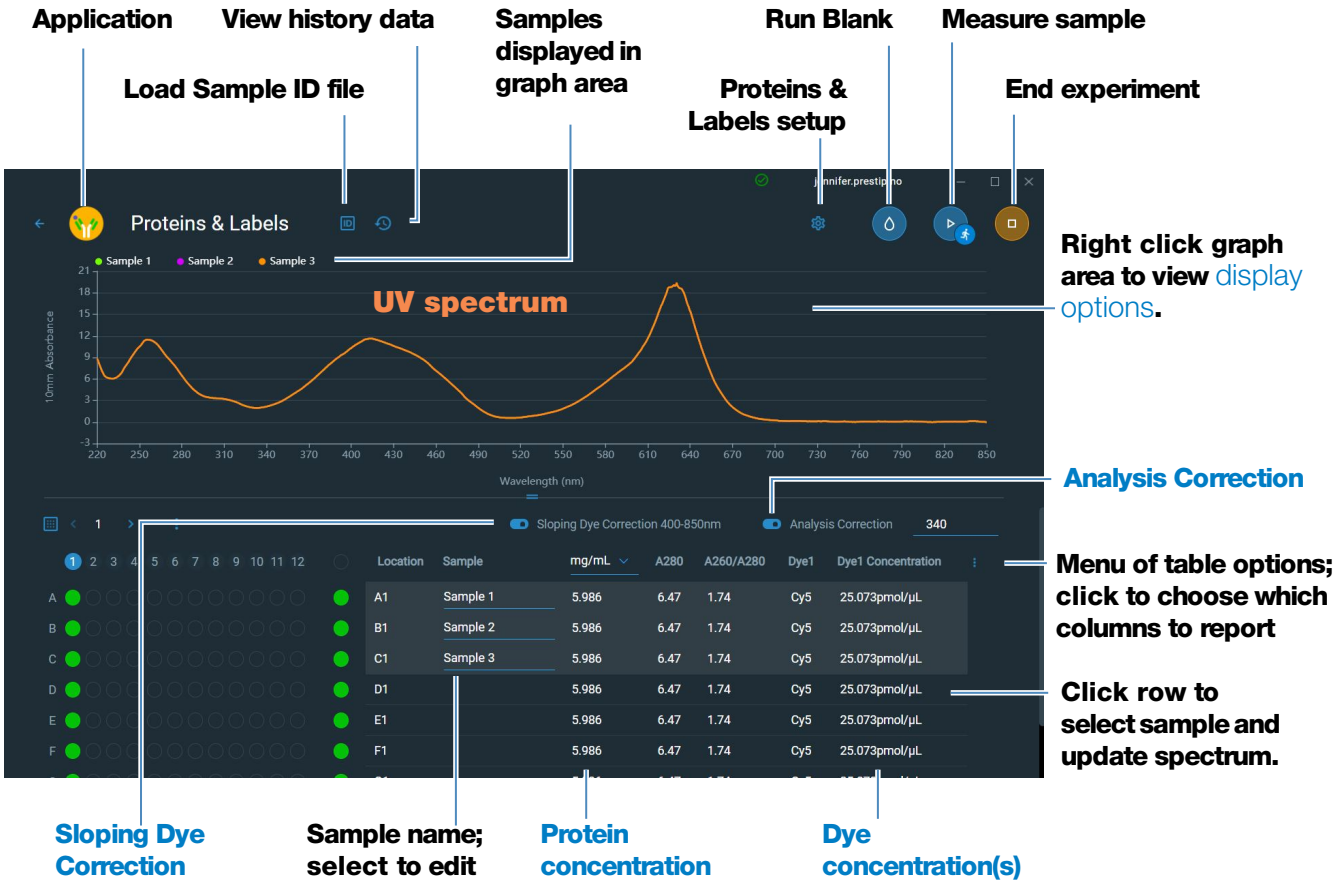
Related Topics

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

Proteins & Labels Reported Results

Proteins & Labels measurement screen

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



Note

- A baseline correction is performed at 340 nm (absorbance value at 340 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

Proteins & Labels reported values

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

Reported values for Proteins & Labels application

- Sample Name
- Creation date
- Protein
- A280
- Sample Type
- Dye 1/Dye 2
- Sloping Dye Correction
- Analysis Correction

Related Topics

- Basic Instrument Operations
- Proteins & Labels calculations

Settings for Proteins and Labels Measurements

To show the Proteins & Labels settings, from the Proteins & Labels measurement screen, select  Proteins & Labels Setup.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type[a]	1 Abs = 1 mg/mL	General reference	Select Sample type for detailed description of each available setting. Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the ε + MW (molar) or ε1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option. **Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.
	BSA	6.7	
	IgG	13.7	
	Lysozyme	26.4	
	Other protein (ε + MW)	user-entered molar extinction coefficient/ molecular weight	
	Other protein (ε1%)	User entered mass extinction coefficient	
Analysis Correction[b]	On or off Enter analysis correction wavelength in nm or use default value (340 nm)	N/A	Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration. **Tip**: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction.
Dye 1/Dye 2 Type[c]	Cy3, 5, 3.5, or 5.5, Alexa Fluor 488, 546, 555, 594, 647, or 660	See Dye/Chromophore Editor for specific values for each dye	Select pre-defined dye used to label sample material, or one that has been added using Dye/Chrom. Editor.
Dye 1/Dye 2 Unit	picomoles/microliter (pmol/µl), micromoles (uM), or millimoles (mM)	not applicable	Select unit for reporting dye concentrations.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sloping Dye Correction[d]	On or off		Corrects dye absorbance measurements for any offset caused by light scattering particulates by subtracting absorbance value of a sloping baseline from 400 nm to 850 nm from absorbance value at dye's analysis wavelength.

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

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

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

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

Related Topics

- Instrument Settings
- Protein Editor
- Dye/Chromophore Editor

Detection Limits for Proteins and Labels Measurements

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

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

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

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

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

$$(200 / 6.7) * 10 = 298.5 \text{ (or } \sim 300)$$

Related Topics

- Detection Limits for All Applications

Calculations for Proteins and Labels Measurements

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

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

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

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

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

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

Available Options for Extinction Coefficient

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

Note: See Sample Type for details.

Measured Values

A280 absorbance

Note: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 nm is zero in the displayed spectra. Also, for micro-volume absorbance measurements, the spectra are normalized to a 10 mm pathlength equivalent.

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

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

Dye absorbance

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

Dye correction

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

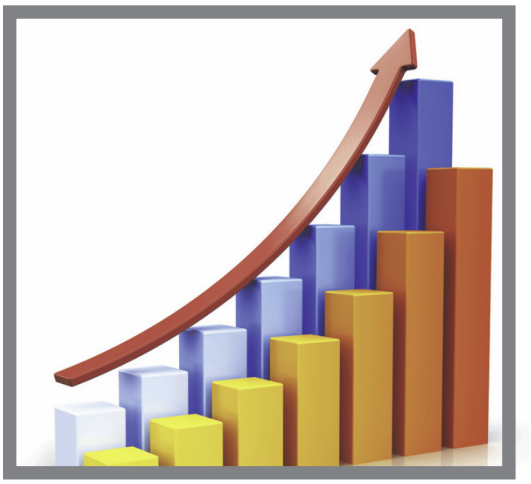
- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **Dye1/Dye2 concentration**. Reported in pmol/µL. Calculations are based on Beer's Law equation using (sloping) baseline-corrected dye absorbance value(s).

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Protein BCA

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



Measure Total Protein Concentration

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

Theory of Protein BCA assay

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

Protein assay kits and protocols

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

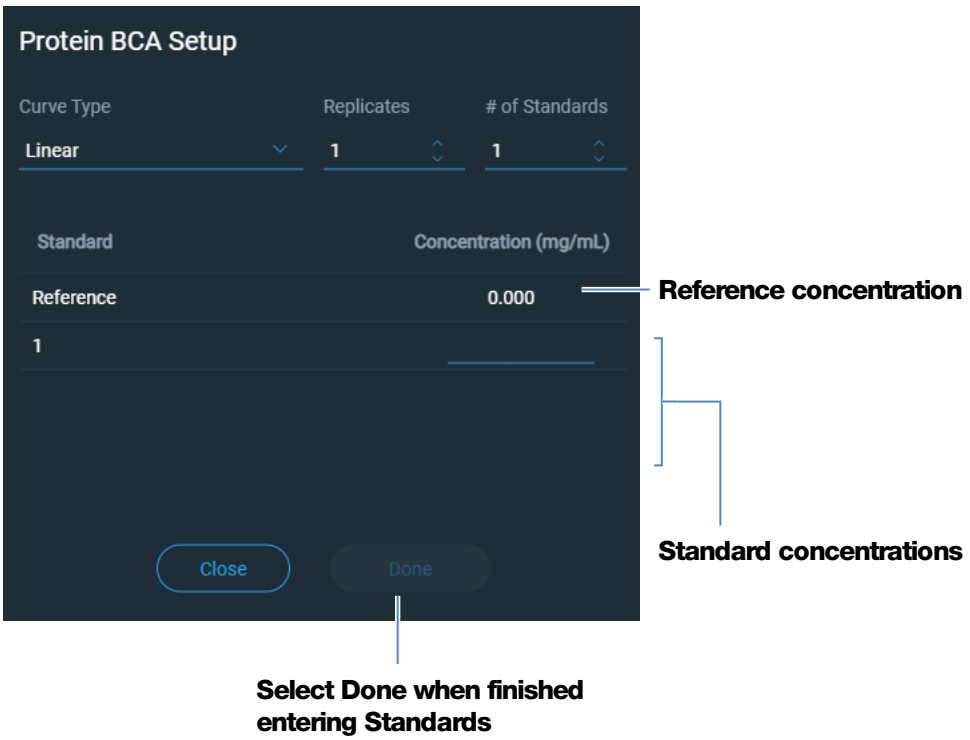
Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop Eight pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than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

Working with standard curves

A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve, or import standard from a previously run experiment.
- To import a Standard curve from a previous experiment, select the  Load Standard icon, select a Standard, and click Load.
- Prepare standards and unknown samples the same way. See the kit manufacturer's guidelines and recommendations.
  - **All reference and standards solutions** should be the same buffer used to resuspend the samples plus the same volume of reagent added to the samples.
  - **First standard** is a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement. This application requires both.)
  - **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard.

- Use the application setup to enter concentration values for the standards and to specify how standards and samples will be measured (number of replicates, etc.).



  - Depending on the Curve Type setting, a standard curve can be generated using two or more standards.
  - The software **requires one reference measurement** and allows **up to 7 standards**.
  - **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest.

- For all colorimetric assays except Protein Pierce 660, **blank the instrument** with DI H_2O (deionized water). For Protein Pierce 660, blank with the reference solution.
- **Measure the reference and all standards** before you start analyzing samples. (After the first sample has been measured, no additional changes are allowed to the standard curve.)

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

Select Standard Curve to see the standard curve you have built.

Here is an example:

Curve type setting

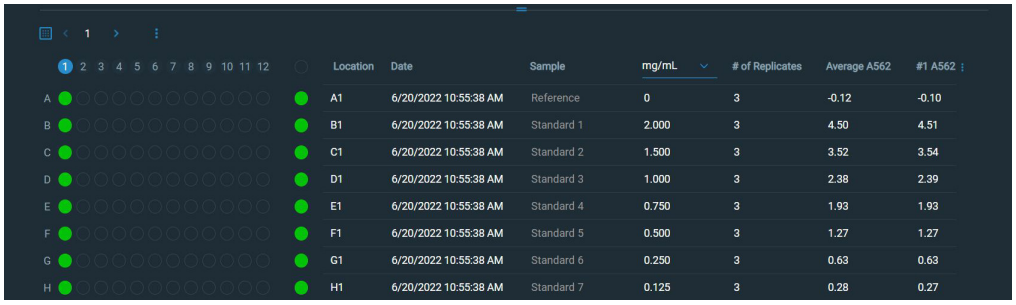
R^2 value (1.0 equals perfect fit)

	Location	Date	Sample	mg/mL	# of Replicates	Average A562	#1 A562
A	A1	6/20/2022 10:55:38 AM	Reference	0	3	-0.12	-0.10
B	B1	6/20/2022 10:55:38 AM	Standard 1	2.000	3	4.50	4.51
C	C1	6/20/2022 10:55:38 AM	Standard 2	1.500	3	3.52	3.54
D	D1	6/20/2022 10:55:38 AM	Standard 3	1.000	3	2.38	2.39
E	E1	6/20/2022 10:55:38 AM	Standard 4	0.750	3	1.93	1.93
F	F1	6/20/2022 10:55:38 AM	Standard 5	0.500	3	1.27	1.27
G	G1	6/20/2022 10:55:38 AM	Standard 6	0.250	3	0.63	0.63
H	H1	6/20/2022 10:55:38 AM	Standard 7	0.125	3	0.28	0.27

Pink circles indicate data points for standards

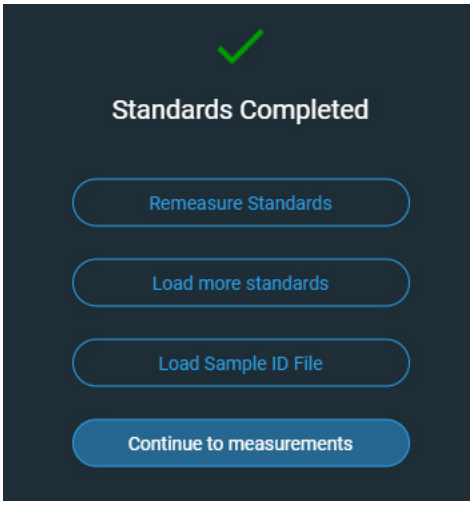
The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; all points lie exactly on the curve).

Standards are listed in the lower half of the screen in the data table.

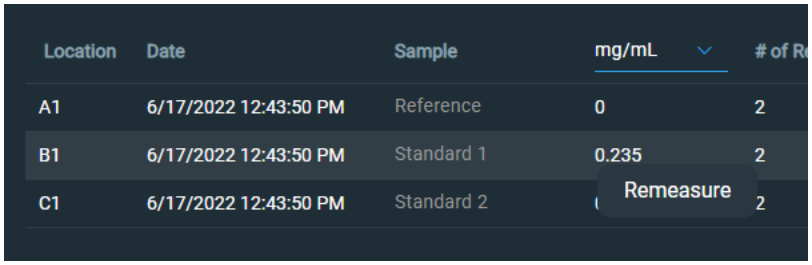


	Location	Date	Sample	mg/mL	# of Replicates	Average A562	#1 A562
A	A1	6/20/2022 10:55:38 AM	Reference	0	3	-0.12	-0.10
B	B1	6/20/2022 10:55:38 AM	Standard 1	2.000	3	4.50	4.51
C	C1	6/20/2022 10:55:38 AM	Standard 2	1.500	3	3.52	3.54
D	D1	6/20/2022 10:55:38 AM	Standard 3	1.000	3	2.38	2.39
E	E1	6/20/2022 10:55:38 AM	Standard 4	0.750	3	1.93	1.93
F	F1	6/20/2022 10:55:38 AM	Standard 5	0.500	3	1.27	1.27
G	G1	6/20/2022 10:55:38 AM	Standard 6	0.250	3	0.63	0.63
H	H1	6/20/2022 10:55:38 AM	Standard 7	0.125	3	0.28	0.27

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



Remeasure standards: select to remeasure any measured standards. Right-click on existing standard(s) and select **Remeasure**.



Load more standards: returns to the setup screen where you can add or edit the concentration value for any standard and then measure the standard.

Load Sample ID File: allows for selection of Sample ID information from an imported sample ID file.

Continue to measurements: continues to sample measurement screen, after which standards can no longer be edited.

- You can add, edit or delete a standard any time before the first sample measurement.

  Add standard:

  – from standards measurement screen, select **BCA Setup**

  – Increment the **#of standards** drop-down and enter the concentration value for the new standard

  – select **Done**

  Edit standard:

  – from standards measurement screen, seelct **BCA Setup**

– select the Concentration field and edit the concentration value

– click **Done**

Delete standard:

– from standards data table, right-click the standard and select **Delete**.

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

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

- After the minimum number of standards has been measured for the selected curve type, the message “Invalid Curve” changes to “Valid Curve.” (This occurs even when additional standards have been defined but not yet measured.) If the “Invalid Curve” message remains after all entered standards have been measured, try:

  – selecting a different curve type

  – remeasuring standards using the correct standard material

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

To measure Protein BCA standards and samples

NOTICE

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

Before you begin...

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

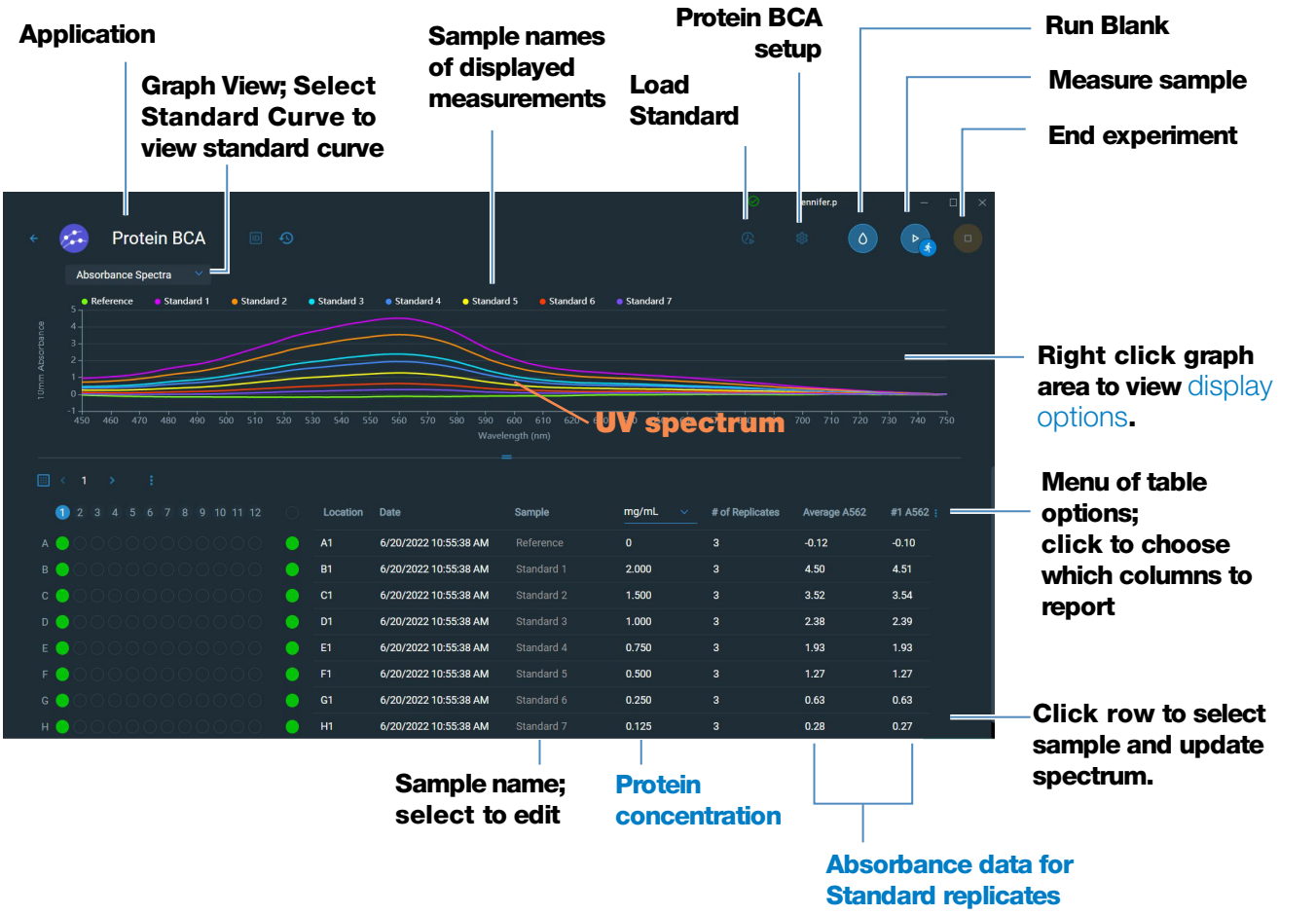
To measure Protein BCA standards and samples

1. From the Home screen, select the **Proteins** tab and select **Protein BCA**.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.
   Tip: For this assay, we recommend setting **Curve Type** to “Linear”.
3. Measure blank:
   - pipette 2 µL DI H_2O onto lower pedestals and lower arm.
   - select **Blank** and wait for measurement to complete.
   - lift arm and clean all pedestals with new laboratory wipe.
4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal. Reference solution should contain none of the standard protein stock, see Working With Standard Curves for details.
   - lower arm to start measurement (select **Measure** if Auto-Measure is off).
   - lift arm and clean all pedestals with new wipe.
   - if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal.
   - lower arm to start measurement (select **Measure** if Auto-Measure is off).
   - lift arm and clean all pedestals with new wipe.
   - if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, select **Load Sample ID File** or **Continue to measurements** (select **Standard Curve** from the view drop-down to view standard curve).
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe.
7. When you are finished measuring samples, select **End Experiment**  .
8. Lift the arm and clean all pedestals with a new wipe.

Protein BCA Reported Results

Protein BCA measurement screen

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. Select “Standard Curve” to view the Standard curve. Below is an example of the measurement screen:



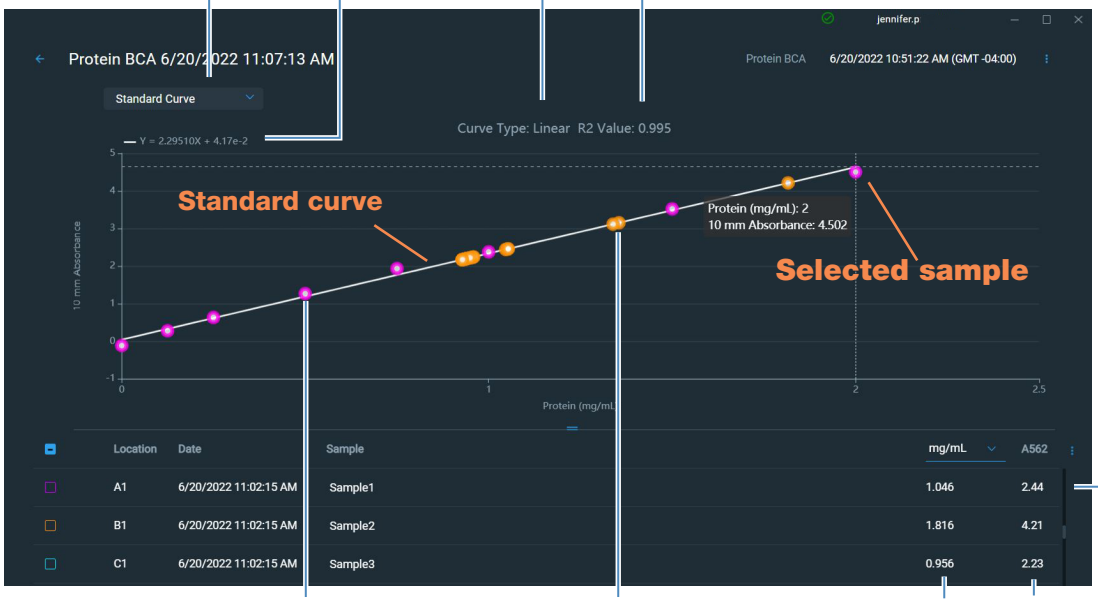
Note

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

Protein BCA standard curve screen

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

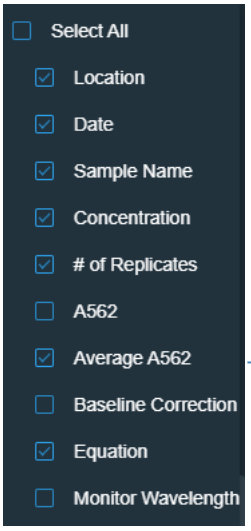
The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Standard curve view

Protein BCA reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:



Date/time measured

Protein conc.

Number of standard replicates

Absorbance at 562 nm

Average absorbance at 562 nm for replicate standard measurements

Baseline correction absorbance

Equation of the standard curve

Additional monitored wavelength

Related Topics

- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein BCA Measurements

To show the Protein BCA settings, click the **Protein BCA Setup** Icon  .

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

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

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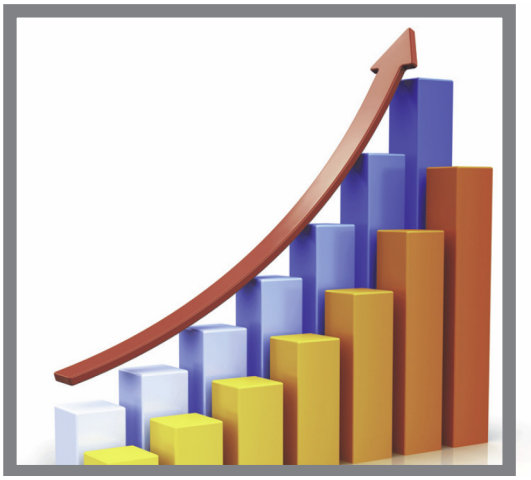
Measure Protein Bradford

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



Measure Total Protein Concentration

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

Theory of Protein Bradford assay

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

To maximize reliability with the Protein Bradford assay:

- **Work quickly and do not allow prepared standards or samples to sit longer than necessary**. Coomassie dye-dye and Coomassie dye-protein aggregates can form particulates with increasing development time, resulting in significant fluctuations in absorbance readings.

- **Measure standards and samples in triplicate** using a new aliquot for each measurement. For pedestal measurements, the total analyte (protein-dye) signal at 595 nm is limited to ~0-0.150A due to the pedestal's 1.0 mm pathlength, the Coomassie dye concentration, and the acidic pH.

Protein assay kits and protocols

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

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

To measure Protein Bradford standards and samples

NOTICE

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

Before you begin...

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

To measure Protein Bradford standards and samples

1. From the Home screen, select the **Proteins** tab and select **Protein Bradford**.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, set **Curve Type** to "2nd Order Polynomial" and **Replicates** to 3.
3. Measure blank:

- pipette 2 µL DI H_2O onto lower pedestals and lower arm.
- select **Blank** and wait for measurement to complete
- lift arm and clean all pedestals with a new laboratory wipe.

4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal. Reference solution should contain none of the standard protein stock, see Working With Standard Curves for details.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe.
   - if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe.
   - if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, select **Load Sample ID File** or **Continue to measurements** (select **Standard Curve** from the view drop-down to view standard curve).
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe,
   - if Replicates setting is greater than 1, repeat measurement.
7. When you are finished measuring samples, select **End Experiment**  .
8. Lift the arm and clean all pedestals with a new wipe.

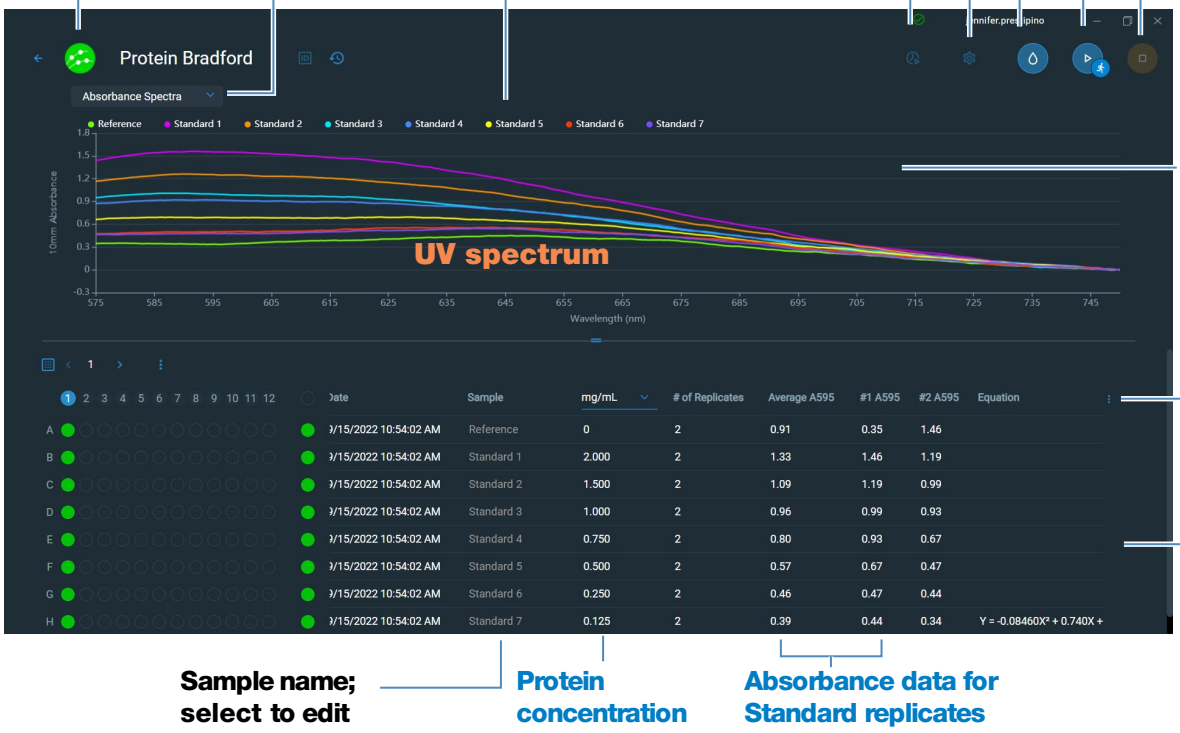
Related Topics

- Working with standard curves
- Best practices for protein measurements

Protein Bradford Reported Results

Protein Bradford measurement screen

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. Select “Standard Curve” to view the Standard curve. Below is an example of the measurement screen:



Date	Sample	mg/mL	# of Replicates	Average A595	#1 A595	#2 A595	Equation
A 9/15/2022 10:54:02 AM	Reference	0	2	0.91	0.35	1.46	
B 9/15/2022 10:54:02 AM	Standard 1	2.000	2	1.33	1.46	1.19	
C 9/15/2022 10:54:02 AM	Standard 2	1.500	2	1.09	1.19	0.99	
D 9/15/2022 10:54:02 AM	Standard 3	1.000	2	0.96	0.99	0.93	
E 9/15/2022 10:54:02 AM	Standard 4	0.750	2	0.80	0.93	0.67	
F 9/15/2022 10:54:02 AM	Standard 5	0.500	2	0.57	0.67	0.47	
G 9/15/2022 10:54:02 AM	Standard 6	0.250	2	0.46	0.47	0.44	
H 9/15/2022 10:54:02 AM	Standard 7	0.125	2	0.39	0.44	0.34	Y = -0.08460X² + 0.740X +

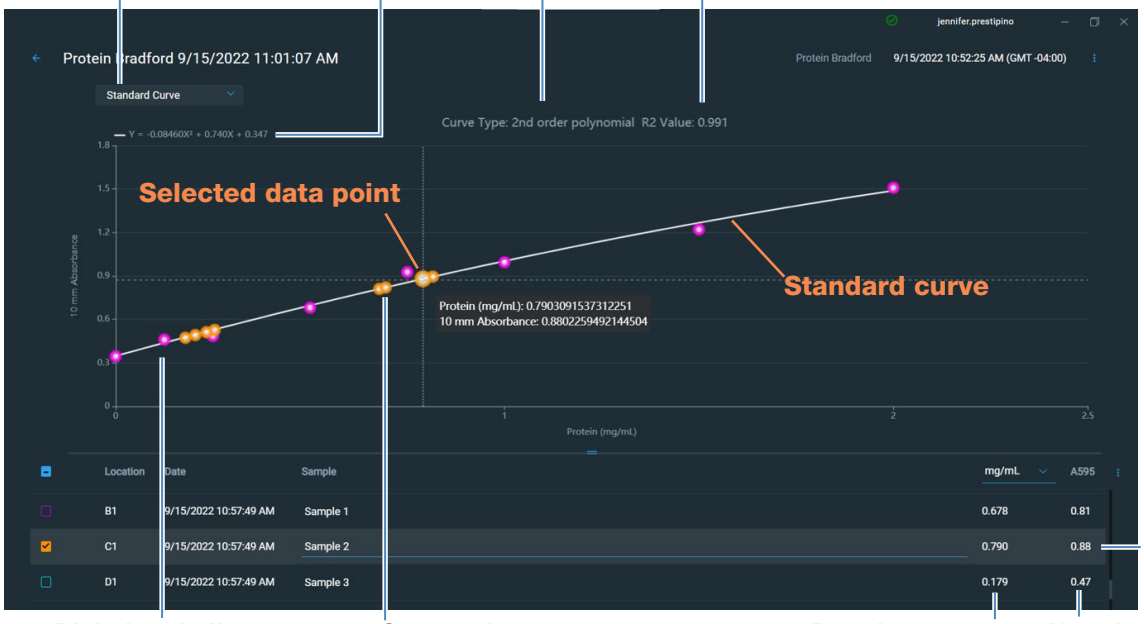
Note

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

Protein Bradford standard curve screen

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

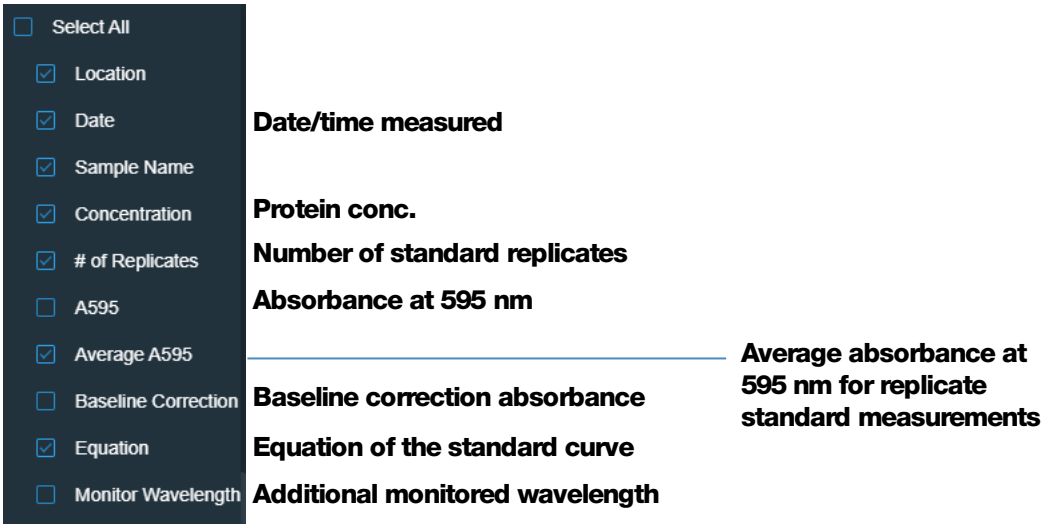
The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Standard curve view

Protein Bradford reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:



Related Topics

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

Settings for Protein Bradford Measurements

To show the Protein Bradford settings, click the  **Protein Bradford Setup** Icon.

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

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

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Measure Protein Lowry

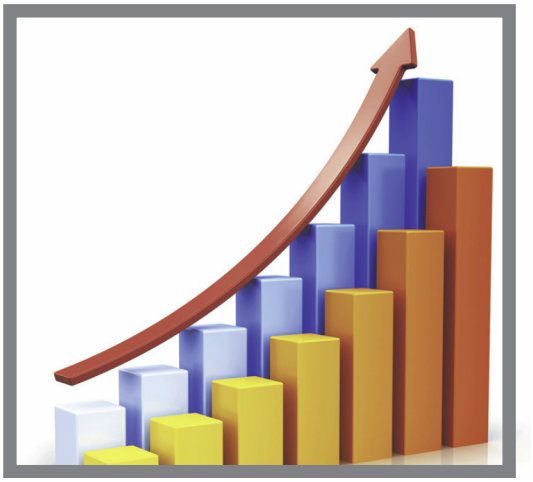
Measures total protein concentration of unpurified protein samples using a Folin-Ciocalteu colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein Lowry assay

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

Protein assay kits and protocols

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

To measure Protein Lowry standards and samples

NOTICE

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

Before you begin...

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

To measure Protein Lowry standards and samples

1. From the Home screen, select the **Proteins** tab and select **Protein Lowry**.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, we recommend setting **Curve Type** to “2nd Order Polynomial.”
3. Measure blank:
   - pipette 2 µL DI H_2O onto lower pedestal and lower arm.
   - select **Blank** and wait for measurement to complete
   - lift arm and clean all pedestals with a new laboratory wipe.
4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal. Reference solution should contain none of the standard protein stock, see Working With Standard Curves for details.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with a new wipe.
   - if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe.

- if Replicates setting is greater than 1, repeat measurement with a new sample aliquot.
- repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples).
- if finished measuring standards, select **Load Sample ID File** or **Continue to measurements** (select **Standard Curve** from the view drop-down to view standard curve).

6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal.
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe.
   - if Replicates setting is greater than 1, repeat measurement.
7. When you are finished measuring samples, select **End Experiment**  .
8. Lift the arm and clean all pedestals with a new wipe.

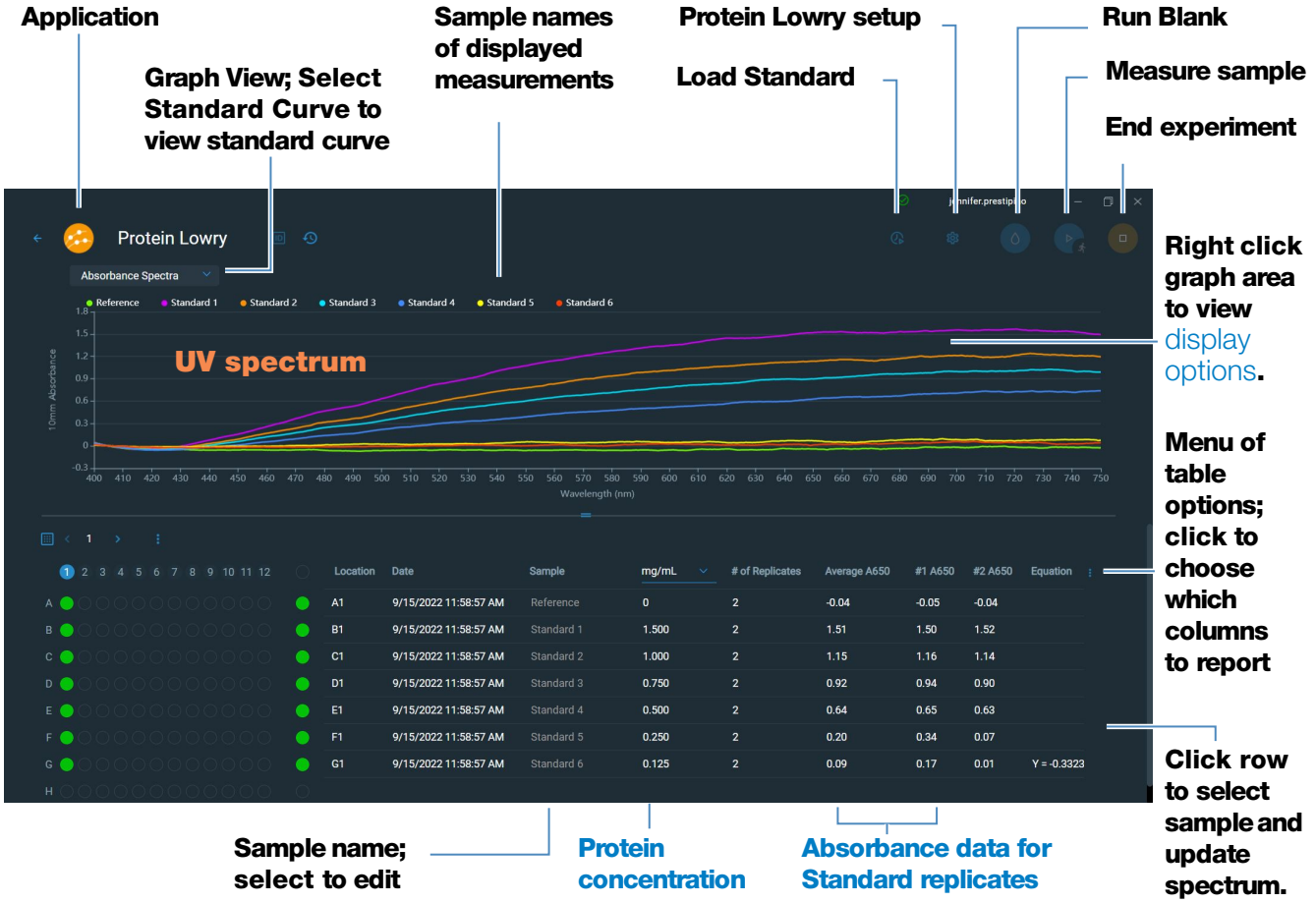
Related Topics

- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Prepare Samples and Blanks

Protein Lowry Reported Results

Protein Lowry measurement screen

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. Select “Standard Curve” to view the Standard curve. Below is an example of the measurement screen:



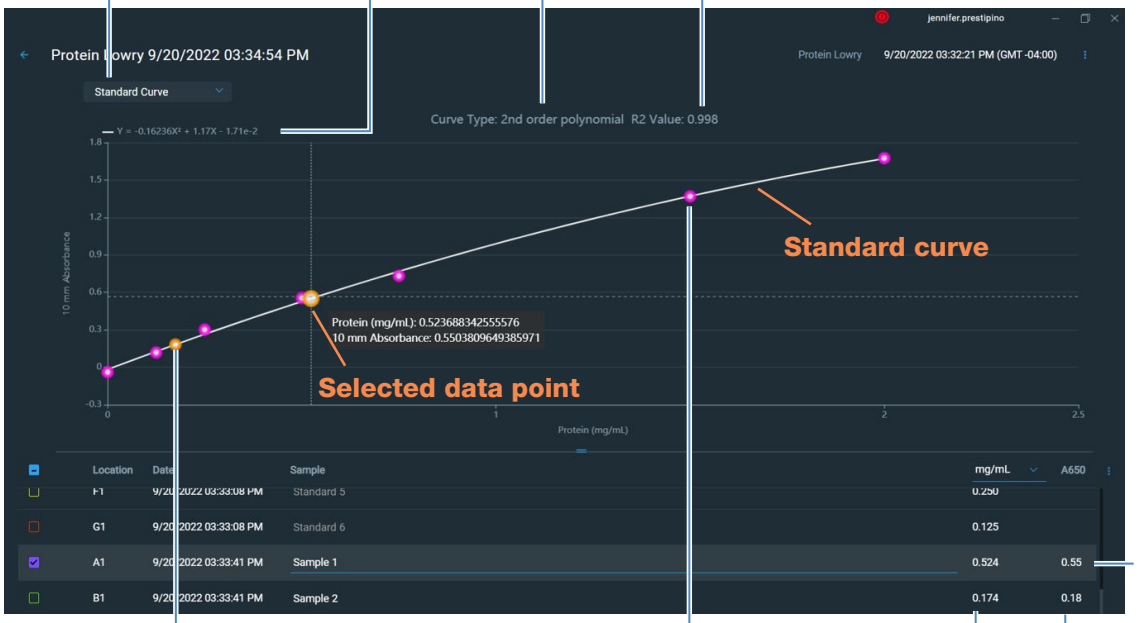
Note

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

Protein Lowry standard curve screen

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

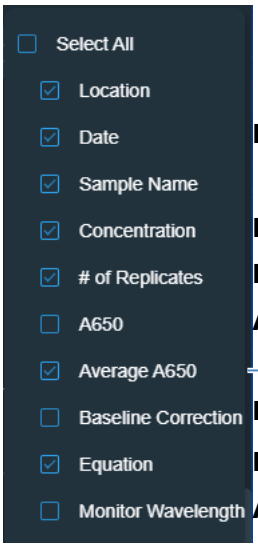
The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Standard curve view

Protein Lowry reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:



Date/time measured

Protein conc.

Number of standard replicates

Absorbance at 650 nm

Average absorbance at 650 nm for replicate standard measurements

Baseline correction absorbance

Equation of the standard curve

Additional monitored wavelength

Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Lowry Measurements

To show the Protein Lowry settings, click the  **Protein Lowry Setup** Icon.

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

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

Related Topics

- Instrument Settings

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Measure Protein Pierce 660

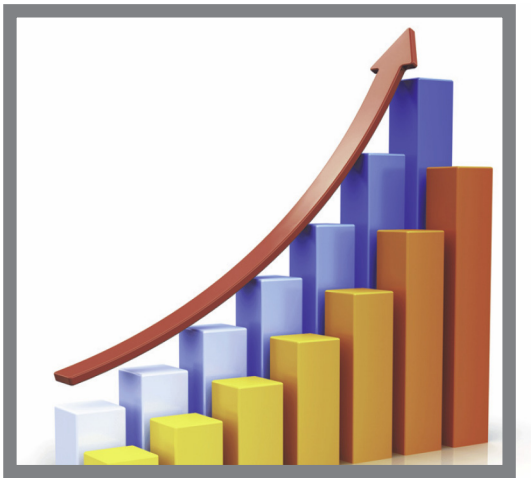
Measures total protein concentration of unpurified protein samples using a proprietary colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein Pierce 660 assay

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

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

Protein assay kits and protocols

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

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

To measure Protein Pierce 660 standards and samples

NOTICE

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

Before you begin...

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

To measure Protein Pierce 660 standards and samples

1. From the Home screen, select the **Proteins** tab and then click **Protein Pierce 660**.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, we recommend setting **Curve Type** to "Linear".
3. Measure blank:
   - Pipette 2 µL reference solution onto lower pedestal and lower arm, Reference solution should contain none of the standard protein stock; see Working With Standard Curves for details.
   - select **Blank** and wait for measurement to complete
   - lift arm and clean all pedestals with a new laboratory wipe,

- Measure reference standard:
- pipette 2 µL reference solution onto pedestal. Reference solution should contain none of the standard protein stock, see Working With Standard Curves for details.
- lower arm to start measurement (or select **Measure** if Auto-Measure is off)
- lift arm and clean all pedestals with a new wipe.
- if Replicates setting is greater than 1, repeat measurement.

4. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal,
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with a new wipe,
   - if Replicates setting is greater than 1, repeat measurement.
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, select **Load Sample ID File** or **Continue to measurements** (select **Standard Curve** from the view drop-down to view standard curve).
5. Measure samples:
   - pipette 2 µL sample 1 onto pedestal,
   - lower arm to start measurement (or select **Measure** if Auto-Measure is off)
   - lift arm and clean all pedestals with new wipe,
   - if Replicates setting is greater than 1, repeat measurement.
6. When you are finished measuring samples, select **End Experiment**  .
7. Lift the arm and clean all pedestals with a new wipe.

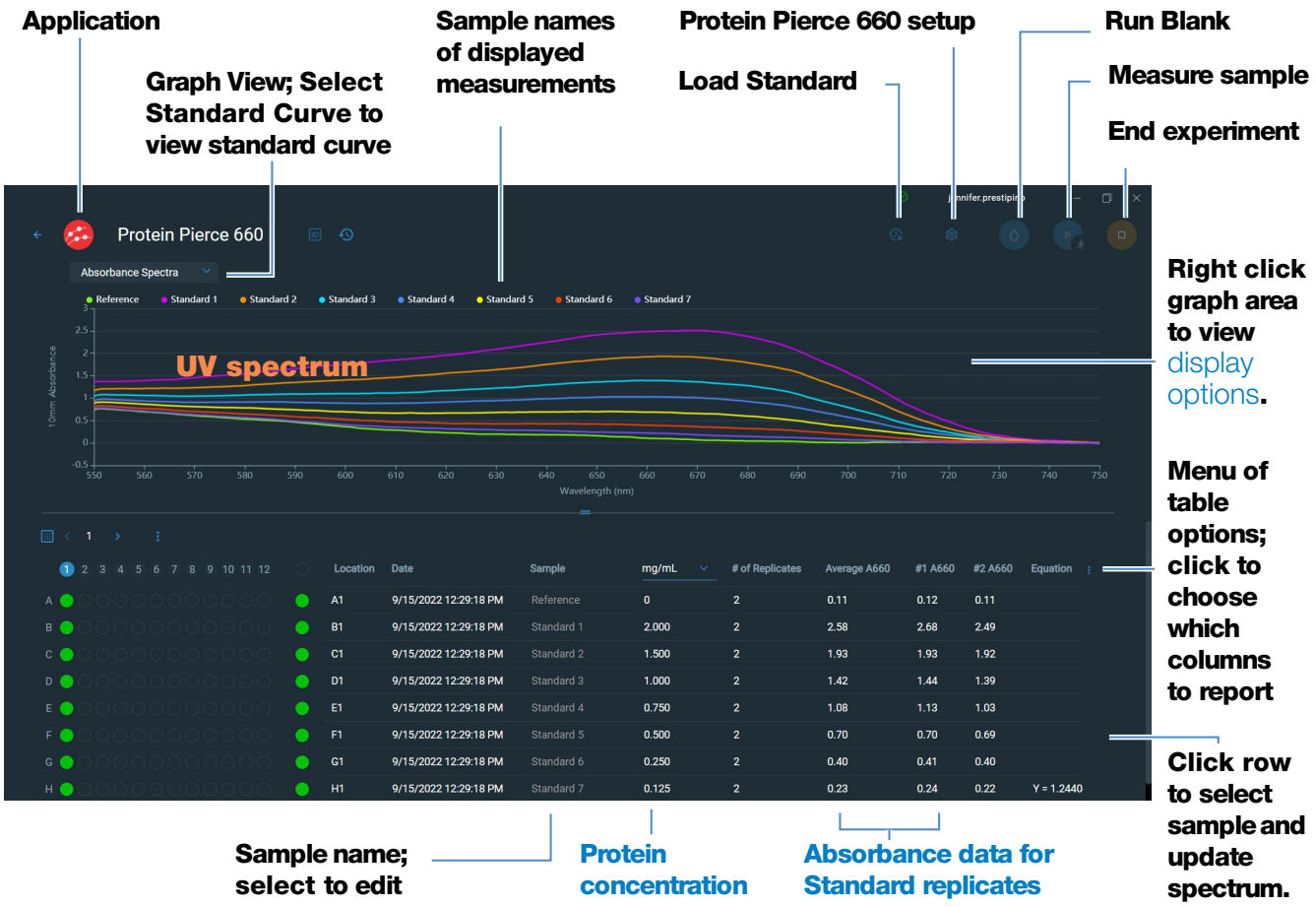
Related Topics

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

Protein Pierce 660 Reported Results

Protein Pierce 660 measurement screen

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. Select “Standard Curve” to view the Standard curve. Below is an example of the measurement screen:



	Location	Date	Sample	mg/mL	# of Replicates	Average A660	#1 A660	#2 A660	Equation
A	A1	9/15/2022 12:29:18 PM	Reference	0	2	0.11	0.12	0.11	
B	B1	9/15/2022 12:29:18 PM	Standard 1	2.000	2	2.58	2.68	2.49	
C	C1	9/15/2022 12:29:18 PM	Standard 2	1.500	2	1.93	1.93	1.92	
D	D1	9/15/2022 12:29:18 PM	Standard 3	1.000	2	1.42	1.44	1.39	
E	E1	9/15/2022 12:29:18 PM	Standard 4	0.750	2	1.08	1.13	1.03	
F	F1	9/15/2022 12:29:18 PM	Standard 5	0.500	2	0.70	0.70	0.69	
G	G1	9/15/2022 12:29:18 PM	Standard 6	0.250	2	0.40	0.41	0.40	
H	H1	9/15/2022 12:29:18 PM	Standard 7	0.125	2	0.23	0.24	0.22	Y = 1.2440

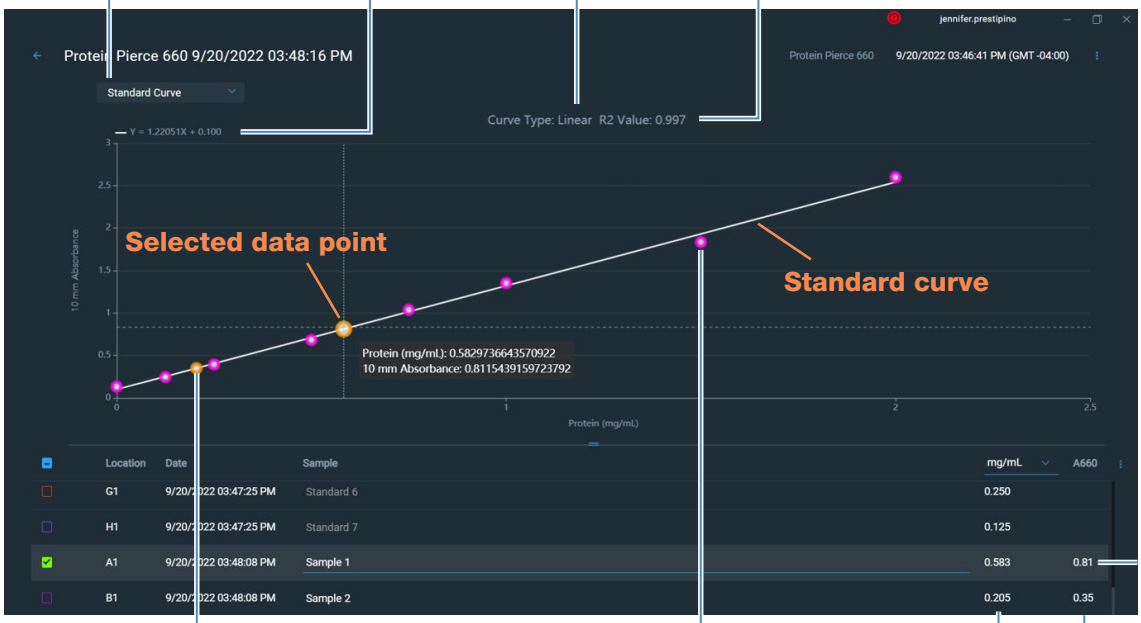
Note

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

Protein Pierce 660 standard curve screen

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

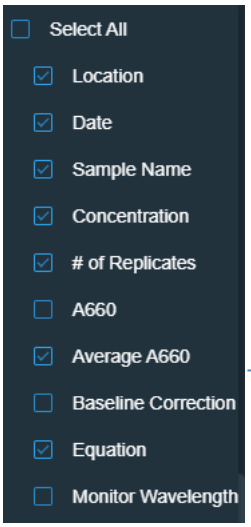
The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Standard curve view

Protein Pierce 660 reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:



Date/time measured

Protein conc.

Number of standard replicates

Absorbance at 660 nm

Average absorbance at 660 nm for replicate standard measurements

Baseline correction absorbance

Equation of the standard curve

Additional monitored wavelength

Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Pierce 660 Measurements

To show the Protein Pierce 660 settings, the  **Protein Pierce 660 Setup** Icon.

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

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

Related Topics

- Instrument Settings

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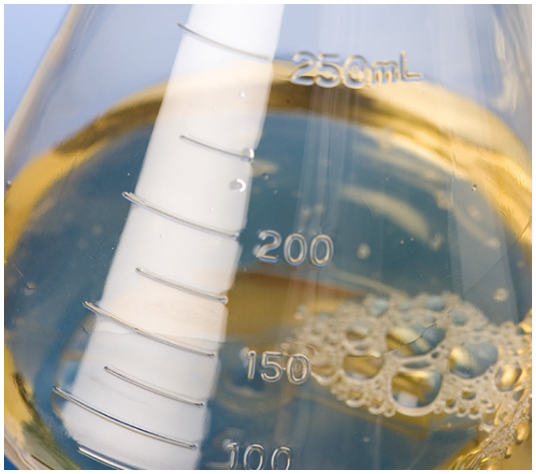
Measure OD600

Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.



Measure OD600

Use the OD600 application to monitor the growth rate of bacterial or other microbial cell cultures by measuring the optical density (absorbance) of the culture in growth media at 600 nm. The Beer-Lambert equation and a user-entered conversion factor are used to correlate absorbance with concentration. Reported concentration values can be used to identify the phase of cultured cell populations, e.g., log or exponential and stationary.

The OD600 application reports cell concentration in cells/mL. A single-point absorbance correction can be used. This application does not require a standard curve.

Note Due to the amount of scattered light present in this assay, absorbance readings are typically very low.

Theory of OD600 application

The OD600 application measures light transmission and uses that value to calculate absorbance. In spectroscopy, transmitted light is defined as any light that is not absorbed by, reflected from and scattered off a sample.

In the case of living cells, most of the incident light is transmitted through the sample rather than scattered, reflected or absorbed. The amount of scattered light is low and can vary from instrument to instrument. As a result, calculated absorbance readings are typically very low.

The calculated absorbance values are used to determine the density of cells in solution in cells/mL. The physical concepts and formulas that relate optical properties of living cells to concentration include:

- Cells, which have a different index of refraction from the surrounding medium, randomly reflect and scatter light out of the incident light path. The amount of scattering is proportional to the density of cells in the sample.
- The Beer's Law equation is used to relate absorbance to concentration. See Calculations for OD600 Measurements for details.
- All measurements should be made on the same type of spectrophotometer and method (i.e., pedestal vs. cuvette) as the amount of scattered light captured varies based on the optical configuration. When using a different spectrophotometer or method, calculate and apply a conversion factor to the reported results. For example, to compare OD readings using the pedestal vs. a cuvette, a conversion factor can be calculated as follows:

  Conversion factor = Cuvette OD/Pedestal OD

Best practices for OD600 measurements

- Ensure the sample is within the instrument's absorbance detection limits.
- Blank with the growth or culture media the cells of interest are suspended in.
- Run a blanking cycle to assess the absorbance contribution of your media solution. If the media solution exhibits strong absorbance at or near the analysis wavelength (600 nm), you may need to choose a different media solution or application. See Choosing and Measuring a Blank for more information.
- Make dilutions as necessary to ensure sample cultures do not exceed the linear dynamic range of the assay before the culture reaches the stationary phase. The linear range depends largely on optical configuration. To determine the linear range:
  - Measure a series of dilutions using a young overnight culture (~16 hrs) of the microbial strain
  - Graph the OD600 measurements against the dilution factor

  The upper detection limit is the measured OD600 value at which there ceases to be a linear correlation between dilution factors and OD600 readings.

- Mix samples gently but thoroughly immediately before taking an aliquot for measurement.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Avoid introducing bubbles when mixing and pipetting.
  - Start the measurement promptly to avoid settling or evaporation.
  - Follow best practices for micro-volume measurements.
  - Use 2 µL sample volume. See Recommended Sample Volumes for more information.
  - For dilute samples that exhibit low absorbance at 600 nm, use an alternative wavelength such as 400 nm to measure absorbance.

To measure OD600 samples

NOTICE

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

Before you begin...

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

To measure an OD600 sample

1. From the Home screen, select the **OD600** tab and select **OD600**.
2. Specify the cell number conversion factor and a additional monitored wavelength or absorbance correction if desired.
3. Pipette 2 µL blanking solution (i.e., the media solution the cells of interest are suspended in) onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

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

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

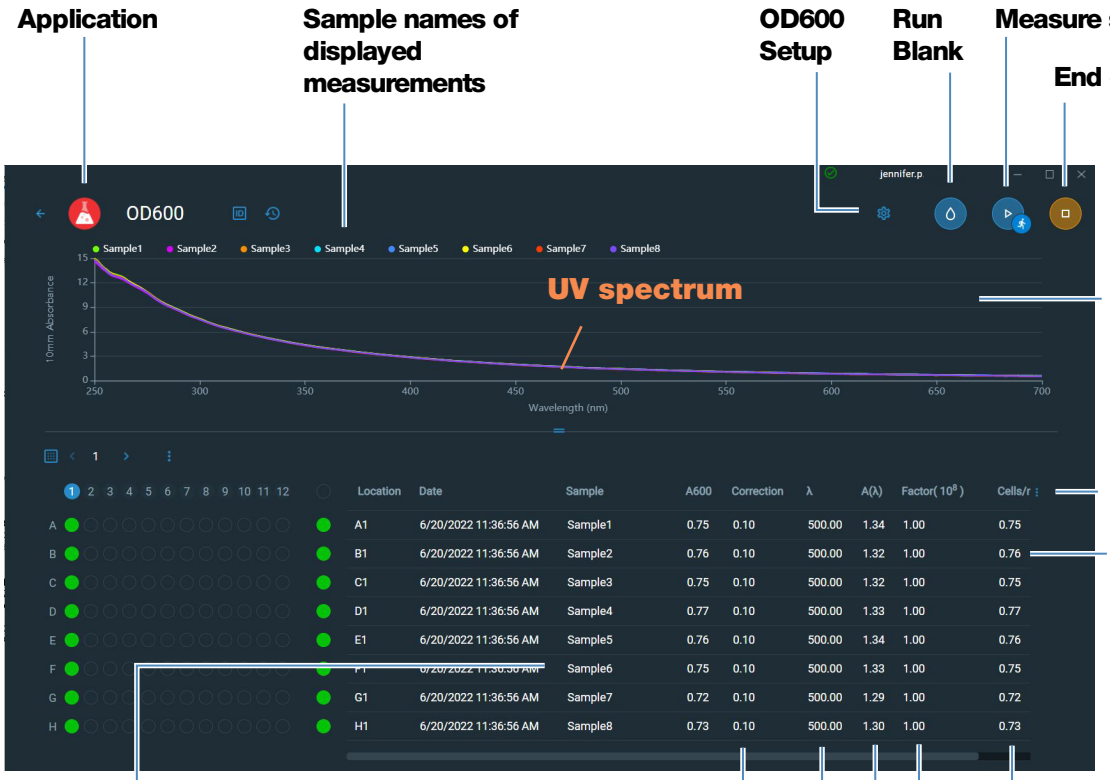
Related Topics

- Measure a Micro-Volume Sample
- Prepare Samples and Blanks
- Basic Instrument Operations

OD600 Reported Results

OD600 measurement screen

The measurement screen shows the UV spectrum and all selected reported values:

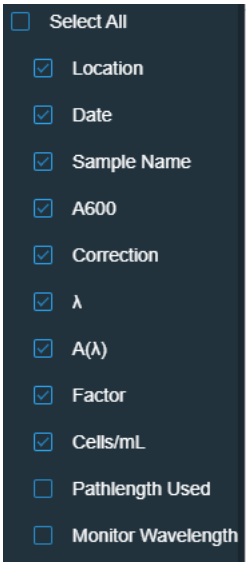


Measurement screen

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

OD600 reported values

Reported values are shown in the Data table. Select which of the reported results is shown in the data table by selecting from the data table options menu. Here are the available reported values:



Date/time measured

Corrected abs. at 600 nm

Absorbance correction

Additional wavelength

Corrected absorbance at additional wavelength

Factor

Cell culture concentration (A600 * Factor)

Related Topics

- Basic Instrument Operations
- OD600 Calculations

Settings for OD600 Measurements

To show the OD600 settings, click the OD600 Setup Icon  .

Setting	Available Options	Description
Absorbance correction	Absorbance value between 0 and 300 A	User-defined absorbance correction. Enter absorbance correction for displayed spectrum. This can be useful, for example, to correct baseline offset caused by any difference between the media solution used to blank the instrument and media used to suspend the cell culture sample, and because scattered light generally produces an offset. Absorbance correction value is subtracted from absorbance values at all wavelengths in sample spectrum. (All displayed absorbance values are corrected values.)

Setting	Available Options	Description
Additional monitored wavelength (λ)	Any wavelength between 250 nm and 700 nm	User-defined wavelength. Enter an additional wavelength to measure if desired (useful for dilute samples that exhibit low absorbance at 600 nm). If an alternative wavelength is specified, use this equation to calculate cell concentration: $c = A(\lambda) * factor(\lambda)$ where: c = analyte concentration in cells/mL A(λ) = UV-visible absorbance at specified wavelength in absorbance units (A) factor(λ) = 1/(ε(λ) * b) in mL/cell-cm where: ε(λ) = molar absorption coefficient (or extinction coefficient) at specified wavelength b = pathlength in cm (1.0 cm for the NanoDrop Eight instruments)
Cell number conversion factor (10^8)	Any number	User-defined factor. Generally accepted factor for measured cell type, or one derived empirically using a solution of study cells at known concentration using the same media. Default value is $1x10^8$ which is the generally accepted factor for most bacterial cell suspensions such as E. coli. **Tip**: The factor is wavelength specific for each cell type and can be affected by the type of media used for the measurements. Ideally, the factor should be determined empirically using a solution of the study cells at a known concentration using the same media.

Related Topics

- Instrument Settings

Calculations for OD600 Measurements

Similar to the nucleic acid applications, the OD600 application uses a modification of the Beer-Lambert equation to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

The OD600 application offers a user-specified factor, to be used in conjunction with Beer's Law to calculate sample concentration. If the factor is known, enter the factor. Otherwise, use $1x10^8$, which is the generally accepted factor for most bacterial cell suspensions such as E. coli.

Calculated cell concentrations are based on the absorbance value at 600 nm, the entered factor and the sample pathlength. A single-point absorbance correction may be applied.

Measured Values

A600 absorbance

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

- Cell culture absorbance values are measured at 600 nm using the normalized spectrum. If no Absorbance Correction is specified, this is the reported A600 value and the value used to calculate cell concentration.
- If an Absorbance Correction is specified, the normalized and (absorbance) corrected absorbance value at 600 nm is reported and used to calculate cell concentration.

A(λ) absorbance

- Normalized and (absorbance) corrected (if used) absorbance value at any specified Additional Monitored Wavelength (λ) is also reported.

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength.
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

Cell concentration. Reported in cells/mL. Calculations are based on Beer-Lambert equation using corrected A600 absorbance value.

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7

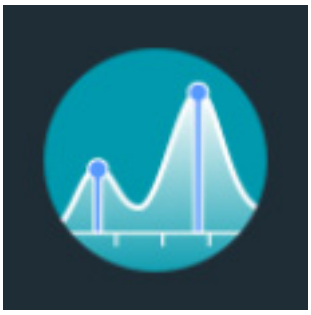
Custom Applications

Use the NanoDrop Eight to perform UV-Vis measurements.

The UV-Vis application allows the instrument to function as a conventional spectrophotometer. Up to 40 wavelengths from 190 nm to 850 nm can be monitored and reported.

Measure UV-Vis

Measures the absorbance of any sample at up to 40 wavelengths across the ultra-violet (UV) and visible regions of the spectrum.



Measure UV-Vis

The UV-Vis application allows the instrument to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 190 nm to 850 nm. Up to 40 wavelengths can be designated for absorbance monitoring and inclusion in the report. Automatic pathlength adjustment and a single-point baseline correction can also be used.

To make UV-Vis measurements

NOTICE

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

Before you begin...

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

To measure a sample using the UV-Vis application

1. From the Home screen, from the **Custom** tab, select **UV-Vis**.
2. Specify up to 40 wavelengths to monitor (or you can specify them later if desired) and whether automated pathlength adjustment, analysis wavelength, and baseline correction will be used.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.

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

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm.

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

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

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

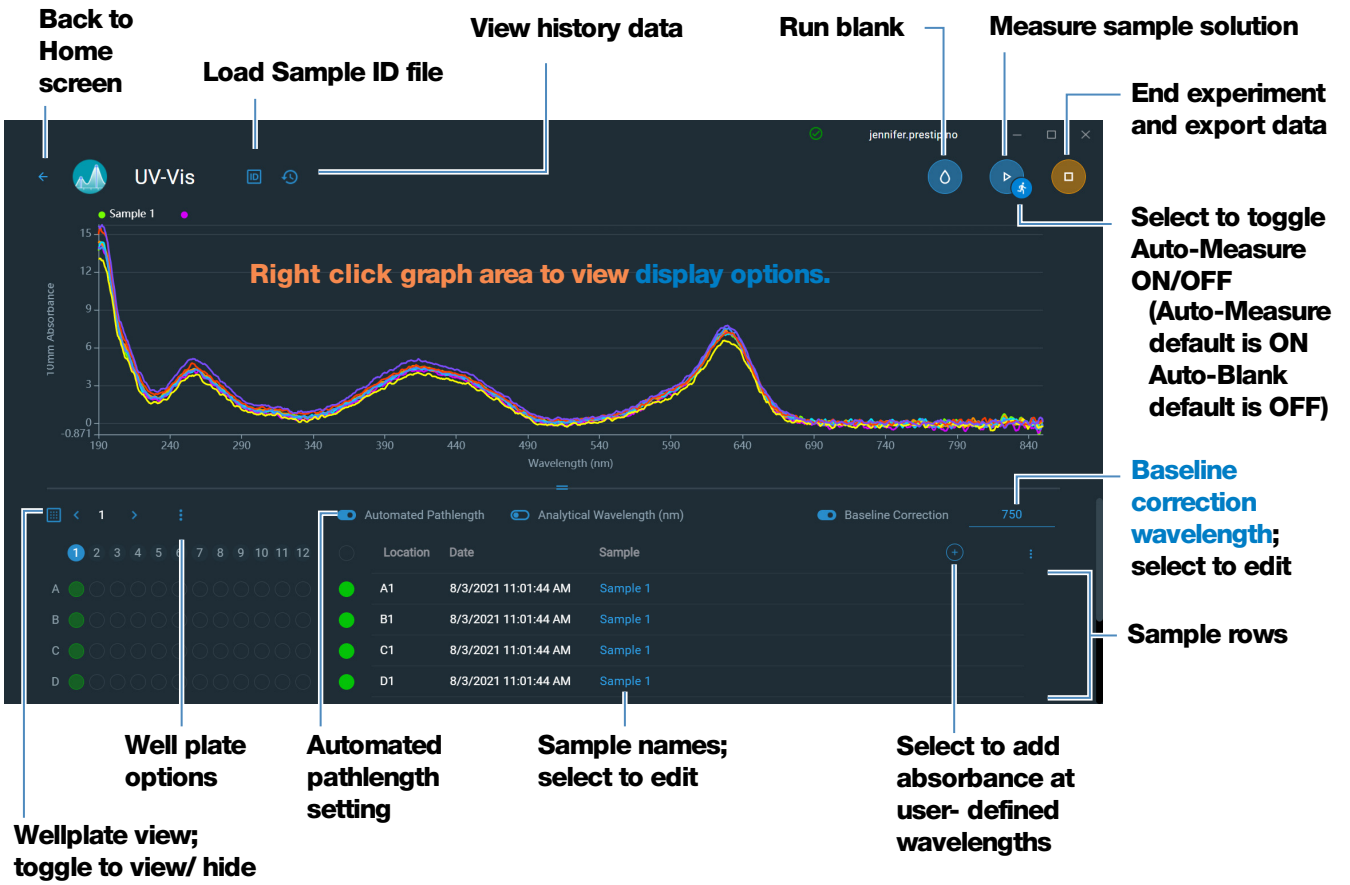
Best practices for UV-Vis measurements

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to re-suspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near an analysis wavelength, you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Ensure samples are homogeneous before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.

UV-Vis Reported Results

UV-Vis measurement screen

For each measured sample, the absorbance spectrum and results are shown:



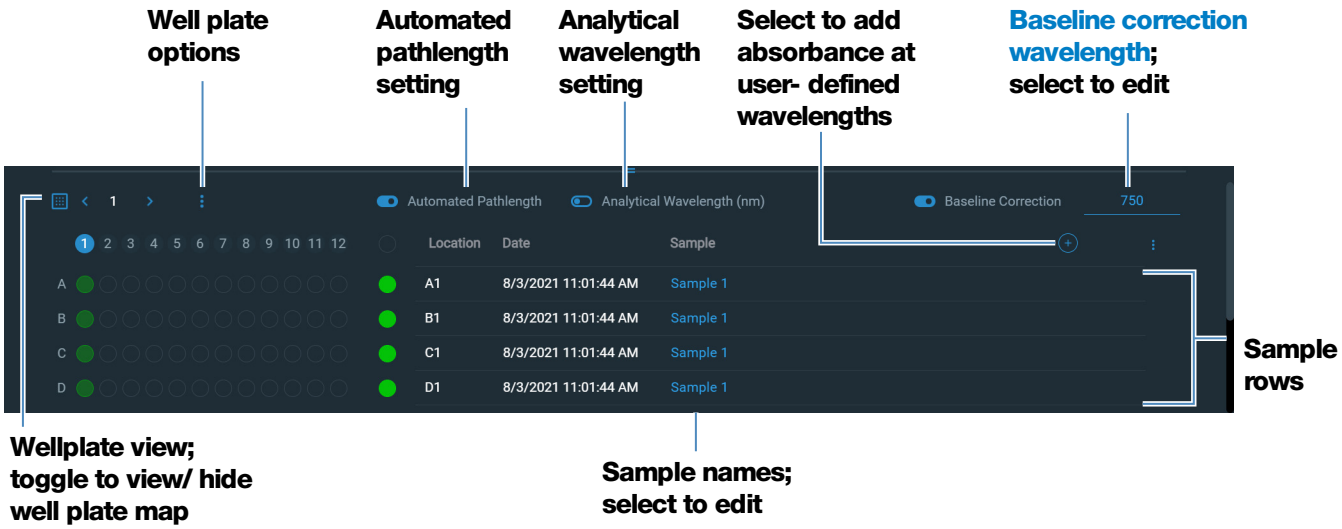
Tips:

Click sample row to select sample and update spectrum
Shift-click multiple sample rows to overlay spectra
Click a sample and hover locations on spectra to view measurement values

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

UV-Vis reported values

The lower half of the measurement screen shows the reported values:



Tips:

Click sample row to select sample and update spectrum
Shift-click multiple sample rows to overlay spectra
Click a sample and hover locations on spectra to view measurement values

Settings for UV-Vis Measurements

To show the UV-Vis settings, from the Home screen, from the **Custom** tab, select **UV-Vis**.

Setting	Available Options	Description
Monitored wavelengths	Enter up to 40 wavelengths between 190 nm and 850 nm	**User-defined wavelengths to be measured and reported at run time**. Absorbance values for the first three entered wavelengths are displayed in the measurement screen. To see absorbance values for 8 monitored wavelengths, swipe left in the measurement screen to show the Data table. To see all monitored wavelengths, press and hold a sample row to show the Sample Details screen (scroll up to display absorbance values for any additional user-defined wavelengths). **Note**: If Baseline Correction is selected, all displayed absorbance values are the corrected values.
Analytical Wavelength	Any wavelength between 190 nm and 850 nm	This is the wavelength the software will use to determine the pathlength selection.
Automated Pathlength	On or Off (affects pedestal measurements only)	**Optional automated pathlength selection**. Allows the software to use the optimal (shorter) pedestal pathlength for high concentration samples to help prevent detector saturation (see Detection Limits for details). • When selected, the shorter pathlength is used when any wavelength has 10 mm equivalent absorbance value of 12.5 or higher. • When deselected, the pedestal pathlength is restricted to 1.0 mm across all wavelengths. **Note**: In either case, displayed absorbance values have been normalized to a 10 mm pathlength equivalent.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (750 nm)	**Optional user-defined baseline correction**. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.

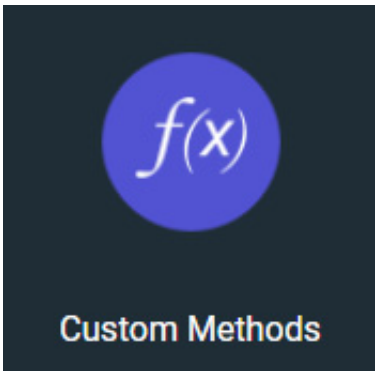
Measure Custom

Runs a custom measurement method created using NanoDrop Eight software.

Measure Custom Method

Delete Custom Method

Reported Results



Measure using a Custom Method

Use the Custom application to run a user-defined method created using the NanoDrop Eight software. For more information, see “Create Custom Method” on page 138.

Custom methods can only be created on a personal computer running the NanoDrop Eight software.

To measure using a custom method

NOTICE

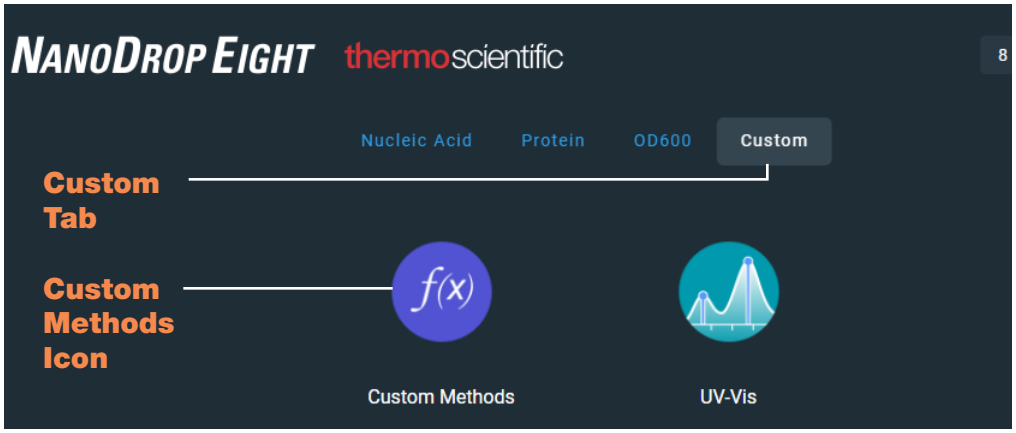
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

Before you begin...

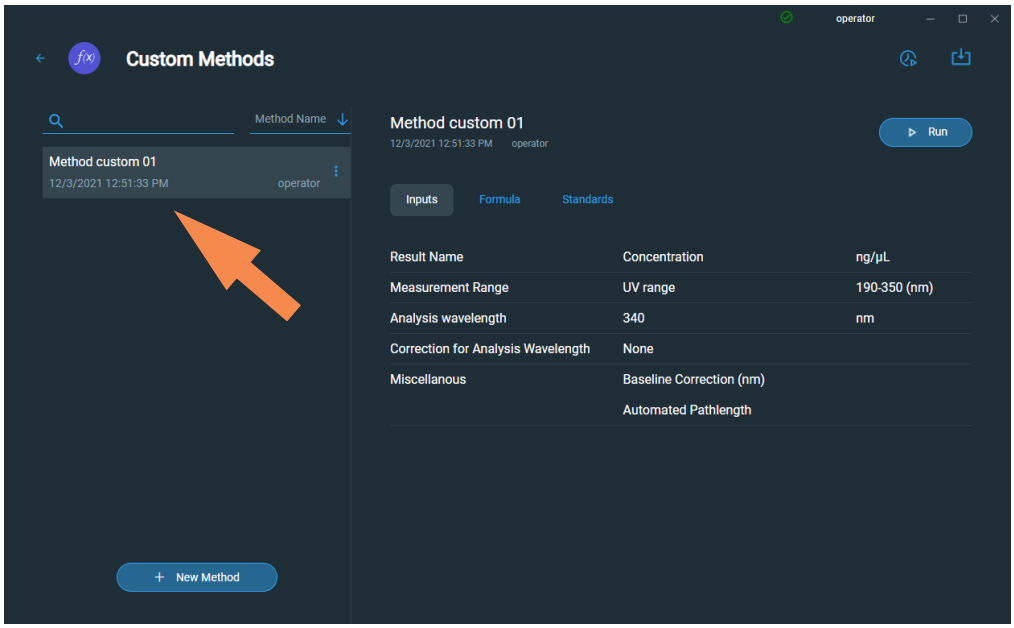
Before taking measurements with the NanoDrop Eight instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

To measure a sample using a custom method

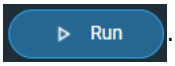
1. From the Home screen, from the **Custom** tab, select **Custom Methods**.



2. In the method selection pane, select the method to run.



Information about the selected method appears in the method details pane.

3. Select 

.
4. Follow the on-screen instructions to measure a sample.

Delete Custom Method

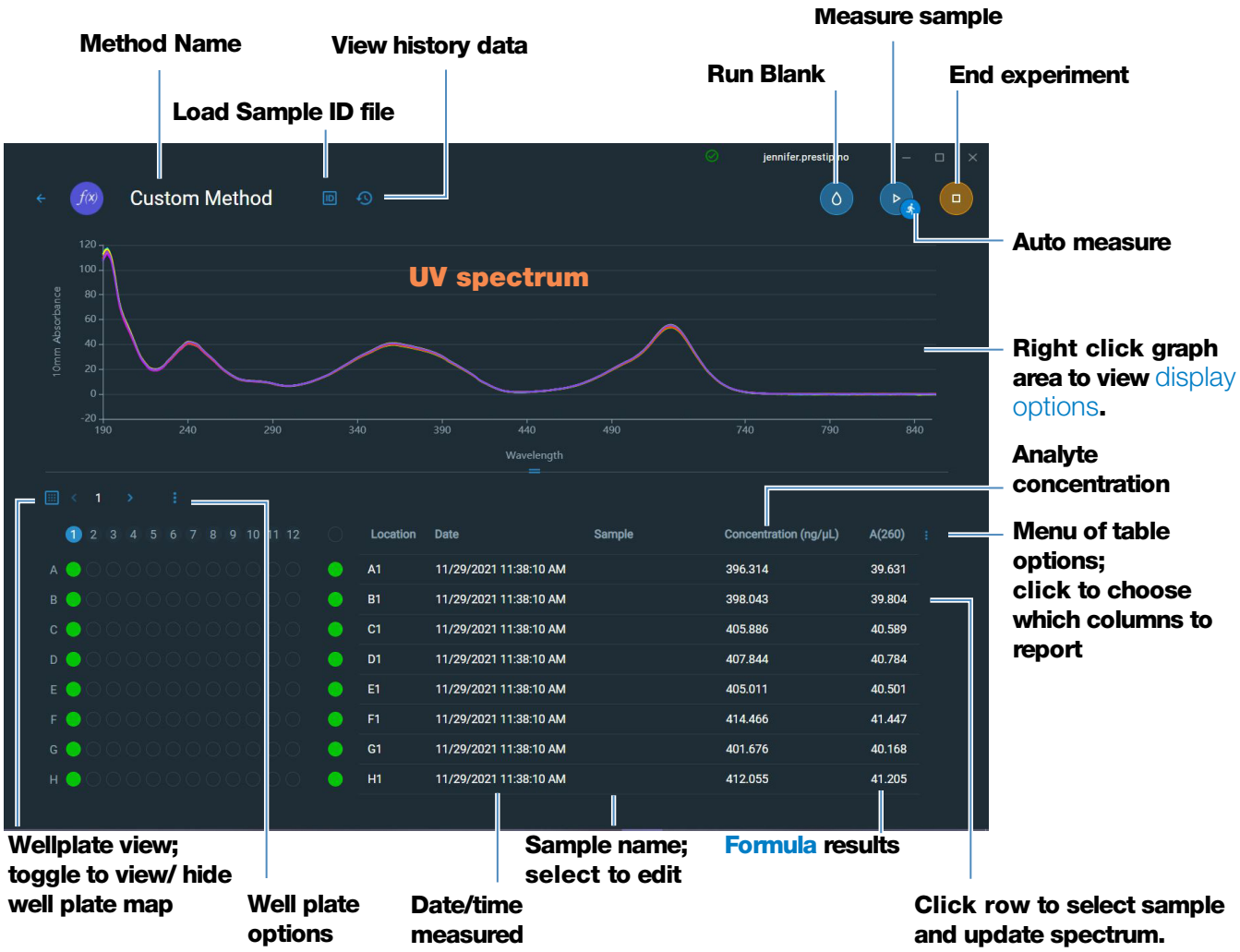
– From Home screen, from the **Custom** tab, select **Custom Methods**.
– In Select Method box, select a method to delete

– From the drop-down menu  select **Delete**

Custom Method Reported Results

Custom method measurement screen

For each measured sample, this application shows the absorbance spectrum and results details. Here is an example:



Custom method reported values

- Result name
- Measurement Range
- Analysis wavelength correction

- Factor or Extinction coefficient
- Standards (Standard curve methods only)
- Baseline correction

Manage Custom Methods

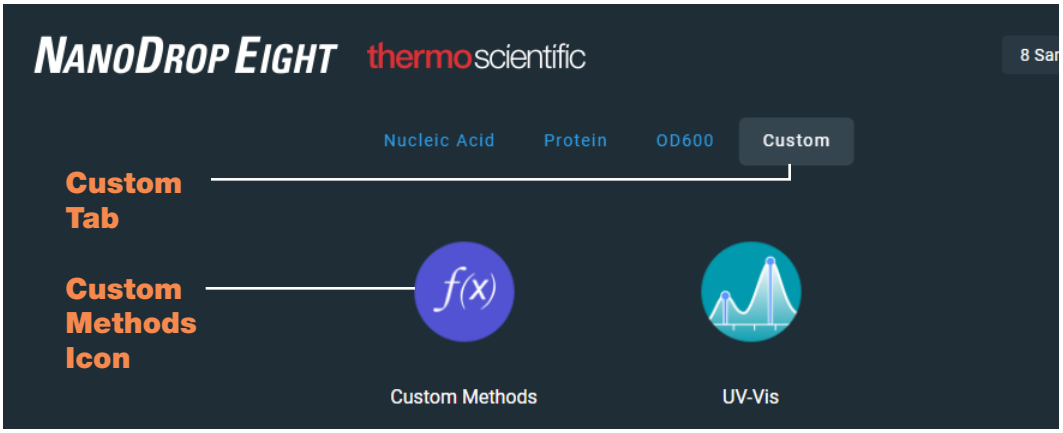
The NanoDrop Eight software is your tool for creating and managing custom methods, which contain user-defined settings that can be used to acquire data with the instrument. Custom methods can be made with or without standards.

Create Custom Method

Create method to be used for sample measurements with user-defined settings.

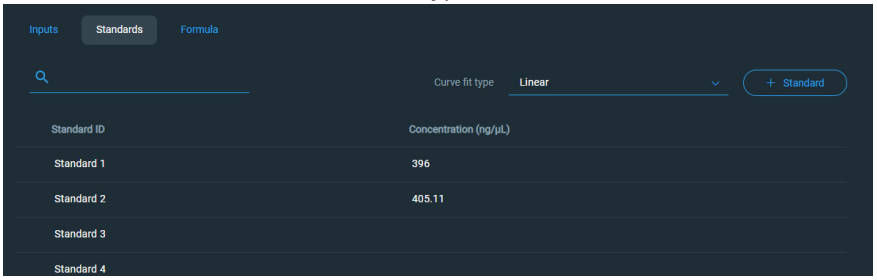
Create new custom method

– From the NanoDrop Home screen, select the **Custom** tab and select **Custom Methods**



– In the Manage Custom Methods screen, select + New Method and choose one of the following:
  – **Formula** (if your method will not have standards)
  – **Standard Curve** (if your method will have standards)
– In the setup window, enter **Method Name**
– Enter detailed **Description** of method, if desired
– Specify how to calculate and report the method results:
  – if method does not have standards, specify factor or extinction coefficient of analyte (enter “1” to report absorbance measurements only)

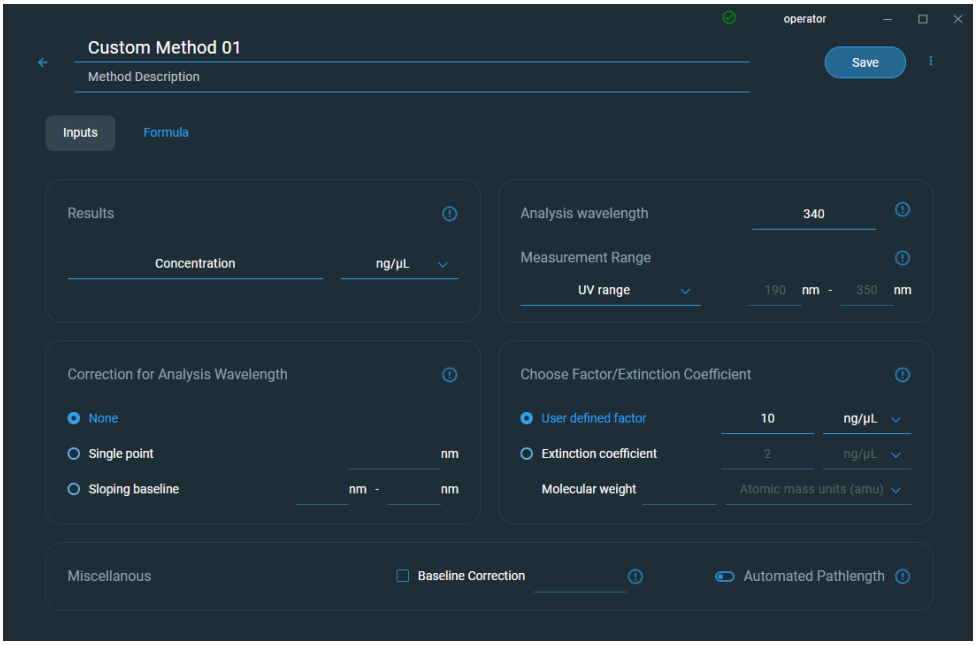
– if method has standards, enter name and concentration of each standard and select the curve fit type.



Alternatively, load a standard curve.

– Enter or choose remaining custom settings as needed

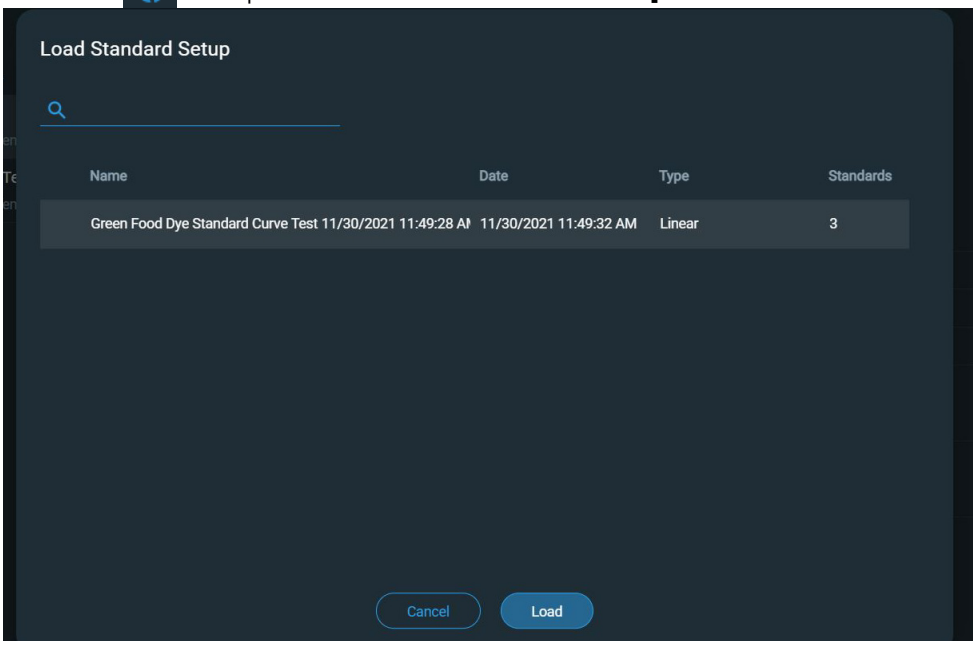
– Choose **Save**



Note Any errors in the method will be listed in red text at the bottom of the method editor screen. Errors must be addressed before the method can be saved.

Load standard curve

– Select  to open the **Load Standard Setup** window.



– Select a standard curve and click **Load**

– The measurement screen opens with the standard curve loaded. You can begin measuring samples

View or edit custom method

– Select **Custom Method** (existing methods are listed in Select Method box along with their type (formula or standards) and Description

– From the Custom Method Management screen, select the method you would like to edit from the list of loaded methods.

– From the drop-down menu  select **Edit**

– View and adjust the method settings as desired

– Select **Save**

Custom method settings

These settings are available for creating custom methods.

Setting	Available Options
Result name	Enter descriptive name for calculated concentration result (for example, “MTT Assay”) and use adjacent drop down list to select appropriate unit. Result name appears as column heading for reported concentration value.
Measurement range	Select spectral range in which method will acquire data. Available options: • Ultra-violet only (190 nm - 350 nm) • Visible only (350 nm - 850 nm) • Ultra-violet and visible (190 nm - 850 nm) • Custom (specify starting and ending point in nanometers) **Notes**: • If a Baseline correction and/or Analysis wavelength correction are used, make sure your selected spectral range includes your specified baseline correction and/or analysis correction wavelength. • For micro-volume absorbance measurements, the spectra are normalized to a 10 mm pathlength equivalent.
Analysis wavelength correction	Use this option to specify absorbance correction at analysis wavelength only. Available options: • **None.** No correction at analysis wavelength. • **Single point**. Enter wavelength for analysis correction. (Absorbance value at specified analysis correction wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.) • **Sloping baseline**. Enter two wavelengths that define sloping baseline for analysis correction. (Absorbance value of sloping baseline at analysis wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.)

Setting	Available Options
Factor or Extinction coefficient at 1 cm pathlength (Formula methods only)	Specify whether to use factor or extinction coefficient to calculate concentration result: • **User-defined factor**. Enter **factor** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how factor is used to calculate sample concentration: $c = (A * f) / b$ where: c = analyte concentration A = absorbance in absorbance units (A) **f** = factor (typically $1/\varepsilon$, where ε = wavelength-dependent molar absorptivity coefficient, or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent) • **Extinction coefficient and molecular weight**. Enter **extinction coefficient** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how extinction coefficient is used to calculate sample concentration: $c = A / (\varepsilon * b)$ where: c = analyte concentration A = absorbance in absorbance units (A) ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent)
	Notes: • Refer to product literature for information about factors and extinction coefficients for specific materials. • To set up a method that reports absorbance measurements only, select Factor or Extinction Coefficient with the factor or extinction coefficient set to “1”. • If specified unit for factor or extinction coefficient is based on mass (such as mg/mL) and specified unit for calculated result is based on molarity (such as pmol/µL) or vice versa, enter **molecular weight** and use adjacent drop down list to select appropriate **unit**.

Setting	Available Options
Standards (Standard curve methods only)	Define the standards: • Enter name and analyte concentration of each standard and a reference, if desired: – Depending on the Curve Type setting, a standard curve can be generated using two or more standards. (The software **allows a reference and up to 7 standards**.) – **All reference and standards solutions** should be in the same buffer used to resuspend the samples plus the same volume of reagent added to the samples. – **First standard** can be a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement.) – **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest. – **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard.
	• Select curve fit type. Specify type of equation used to create standard curve from standard concentration values. Available options: – Linear: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement ans at least one standard) – 2^{nd} order polynomial: Draws the 2^{nd} order least squares polynomial using all measured standards (requires reference measurement and at least standards) – 3^{rd} order polynomial: Draws the 3^{rd} order least squares polynomial using all measured standards (requires reference measurement and at least three standards)

Setting	Available Options
Analysis wavelength (Standard curve methods only)	Monitor absorbance at specified wavelength (enter the wavelength in nanometers). **Note**: The specified wavelength must fall within the selected measurement range. The measurement results or the concentration will be calculated automatically using the absorbance value at the specified wavelength and applying the selected method type (factor or standard curve).
Baseline correction	Select this option to correct offset caused by light scattering particulates by subtracting the absorbance at a specified baseline point. Then specify wavelength for baseline correction. **Note**: Software subtracts absorbance value at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.
Automated pathlength	Affects micro-volume measurements only. • When Automated Pathlength is selected, software selects the optimal pathlength (between 1.0 mm and 0.1 mm) based on sample absorbance at the analysis wavelength. For example, when sample absorbance at the analysis wavelength is less than or equal to 12.5 (10 mm pathlength equivalent), the optimal longer pathlength is used. When sample absorbance is greater than 12.5, the optimal shorter pathlength is used. Recommended for samples that are highly absorbing at the analysis wavelength. (This option may cause reduced sensitivity when the sample spectra have a large absorbance peak that is not at the analysis wavelength.) **Note**: When the analysis wavelength is between 190 nm and 219 nm, the optimal longer pathlength is used when sample absorbance is less than or equal to 10 (10 mm pathlength equivalent), and the optimal shorter pathlength is used when sample absorbance is greater than 10. • When Automated Pathlength is deselected, the software uses a 1 mm pathlength regardless of the sample absorbance. This can cause detector saturation (resulting in jagged peaks) for highly absorbing samples (e.g., ~15 A at 10 mm pathlength equivalent).

Setting	Available Options

Formula table (optional)	Use the Formula table to specify additional reported results, such as a purity ratio, for each sample.
	
	Available options:
	
	• **Predefined**. Select from a list of predefined formulas, which can be used as is or edited, and choose **Add**. The predefined formula is listed in the Formula Table.
	
	• **Add**. Create formula for current method. Available options:
	
	  • **Formula Name**. Enter a name for the formula. After a measurement, the name is reported in Data Table and Sample Details screens.
	
	  • **Formula**. Enter valid formula (see below for rules and examples). After a measurement, the measured or calculated value is reported in Data Table and Sample Details screens.
	
	  • **Unit**. Enter unit for reported result. After a measurement, the unit is reported in Data Table and Sample Details screens.
	
	• **Edit**. Edit selected formula for current method.
	
	• **Delete**. Delete selected formula from current method.

Formula rules	Custom formulas can include the following operators and functions: • **Path()**. Returns sample pathlength in cm. • **A(nm)**. Returns sample absorbance at specified wavelength (for example, enter A(650) to add the measured absorbance at 650 nm to your equation). • **Operators**: + (add), - (subtract), * (multiply), / (divide). • **Functions**: Log(x), Pow(x,y). **Notes**: Follow these additional rules for all languages: • Use period “.” decimal separators for floating point and double-floating point numbers. • Use comma “,” list separators (for example, “POW(2,8)”). • Do not use comma “,” group separators for large numbers (for example, enter 1000 rather than 1,000).

Copy Custom Method

To create a custom method that is similar to an existing one, open the existing method, make your changes, then select **Save As** and enter a new name.

Copy custom method

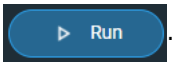
- From the Custom Methods screen, select a custom method
- From the drop-down menu  choose **Edit**
- Enter new **Method name** and **Description**
- Select **Save As**
- Enter a filename for the method and click **Save**

You can now select the saved method and edit the **Description** and settings.

Run Custom Method

To run a custom method, first create or import a custom method.

Run custom method

- From the Custom Methods screen, select a custom method
- Select 

.
- Follow the on-screen instructions to measure a sample.

Export Custom Method

Export a custom method in order to run it and store the measurement results on another PC for use with another NanoDrop Eight instrument.

- From the Custom Methods screen, select a custom method
- From the drop-down menu  , choose **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported)
- Choose **Save** (method is exported to method file (*.method filename extension) in proprietary format)

Import custom method

You can import an existing custom method file to edit the method settings.

- From the Custom Methods screen, choose **Import** 
- Locate and select ".method" file
- Choose **Open** (imported method is added to end of Select Method list)

Edit custom method

Edit a custom method in order to change the method settings.

- From Custom Methods screen, select a custom method from the list of available methods
- From the drop-down menu  , choose **Edit**
- Edit method settings as desired
- Choose **Save**

Delete custom method

- From Custom Methods screen, select a custom method from the list of available methods
- From the drop-down menu  , choose **Delete**
- After the confirmation message, choose **Yes**

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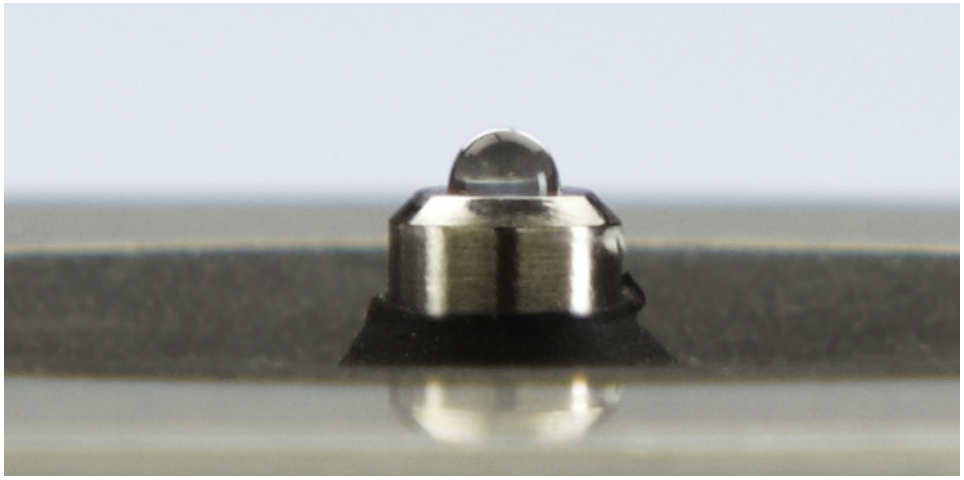
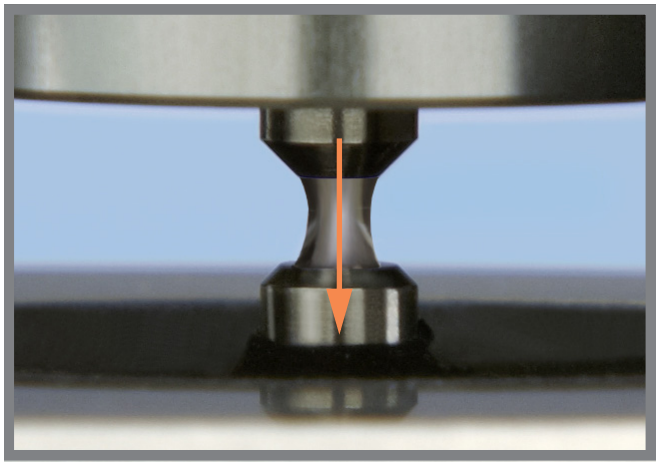
8

Learning Center

Contents

Micro-Volume Sampling—How it Works

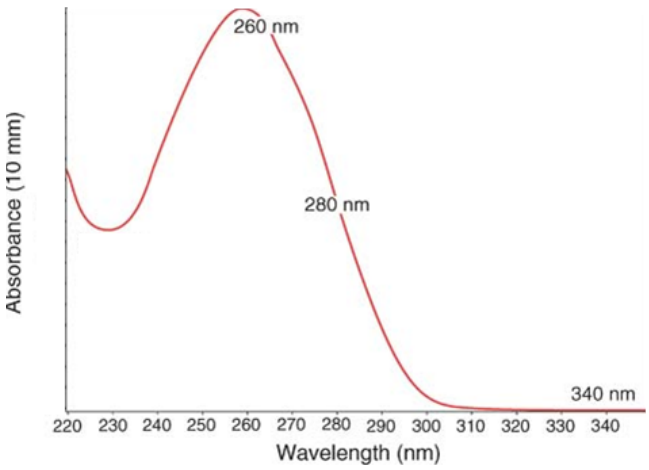




Surface Tension

The NanoDrop Eight spectrophotometer uses surface tension to hold a small volume of sample between two pedestals (upper and lower). The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

A fiber optic cable embedded in the upper pedestal leads to a xenon light source. A second cable embedded in the lower pedestal leads to a detector. When the instrument arm is down, the sample forms a liquid column, essentially bridging the gap between the two fiber optic cables.



Absorbance Spectrum

The light passes through the liquid column to the detector, which generates a spectrum of absorbance versus wavelength. The spectrum shows the amount of light absorbed by the molecules of the sample at each measured wavelength.

Note: To prevent evaporation, which affects measurement accuracy, close the arm quickly after you finish loading a sample or blank.

The example at the left shows a typical absorbance spectrum taken of a nucleic acid sample. The spectrum is measured from 190 nm to 850 nm. The displayed range may vary for each application.

Sample Absorbance

$$\text{Absorbance} = -\log\left[\frac{\text{intensity}_{\text{sample}}}{\text{intensity}_{\text{blank}}}\right]$$

When the instrument is blanked, a reference spectrum is taken of the blanking solution and stored in memory. For each sample measurement, the sample intensities along with the blank intensities are used to calculate the total absorbance of the sample according to the equation at the left.

Beer-Lambert equation

$$A = \varepsilon \cdot b \cdot c$$

where:

A = absorbance in absorbance units (A)

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

b = pathlength in cm

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

Sample Concentration

The Beer-Lambert equation (Beer's law) shown at the left is used to correlate sample absorbance with concentration.

The pathlength is the distance between the two pedestals, which varies in real time during each measurement. This auto-ranging pathlength technique produces accurate concentration results over a wide dynamic range.

Baseline Correction

For some applications, the instrument can be set up to apply a baseline correction to each measurement to minimize any offset caused by light scattering particulates in the sample spectra. The correction subtracts the absorbance value at a reference wavelength that is close to zero from the absorbance value at each wavelength across the spectrum, essentially "anchoring" the spectrum to zero absorbance units at the reference wavelength.

Set Up the Instrument

Connect Power

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

Connect the provided power cord to a grounded wall outlet. See "Power Cords" on page 222 for more information.

Connect to a Computer

Connect the USB cable to the NanoDrop Eight and to an available USB port on your PC.

Operating Specifications

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

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

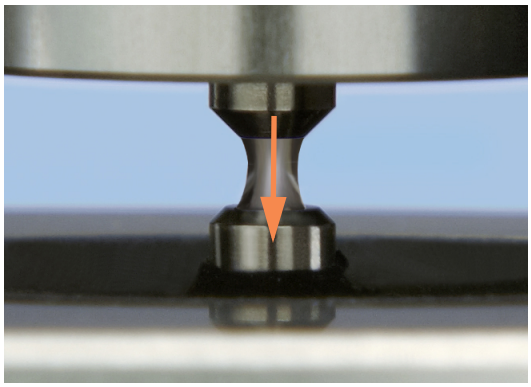
Locate the instrument away from air vents and exhaust fans to minimize evaporation.

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

After the instrument is installed, you can leave it turned on.

Measure a Micro-Volume Sample

The NanoDrop Eight spectrophotometer uses surface tension to hold a small volume of sample between two pedestals. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions. See "Micro-Volume Sampling—How it Works" on page 150 for details.



Supplies needed

- NanoDrop Eight spectrophotometer
- lint-free laboratory wipes
- calibrated precision pipettor
- sample material resuspended in appropriate buffer solution (see Preparing Samples)
- pure buffer solution for blanking instrument (see Choosing and Measuring a Blank.

Best practices for micro-volume measurements

Cleaning pedestals for daily operation

- Before first measurement, clean all pedestals with a new laboratory wipe.
- Run a blanking cycle to verify pedestals are clean.
- After each measurement, clean all pedestals with new wipe to prevent carryover.
- After each set of measurements, clean pedestals with DI H2O (see Clean pedestals between users)
- Recondition pedestals periodically to maintain their hydrophobic property.

Pipetting Samples

- Position the instrument at an angle for optimal use of the pipette guide.
- Use calibrated precision pipettor (0–2 µL volume range) with well-fitting, low-retention precision tips to apply sample material to instrument for measurement. If using low accuracy (0-10 µL) pipettor, use 2 µL sample volumes.

- Filter tips are not recommended, as filter particulates can impact absorbance measurements at 230 nm.
- Use recommended sample volumes to ensure proper liquid column formation.
- Use new tip for each blank and sample aliquot.
- Use new aliquot of sample for each measurement.
- When the measurement is complete, open the sampling arm and wipe the samples from both the upper and lower pedestals using a soft laboratory wipe.

If solvents are used, make sure they are compatible with the pedestals. (see "Compatible Solvents" in Hazardous Materials).

Recommended sample volumes

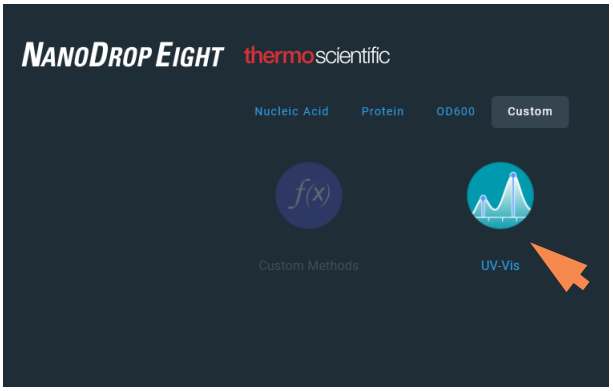
Application	Sample Volume
Nucleic acid (aqueous solution)	1 µL[a]
Purified protein	2 µL
Other protein applications such as Bradford or BCA	2 µL
Microbial cell suspensions	2 µL

[a] Use 2 µL for samples that contain materials that may reduce surface tension such as a surfactant.

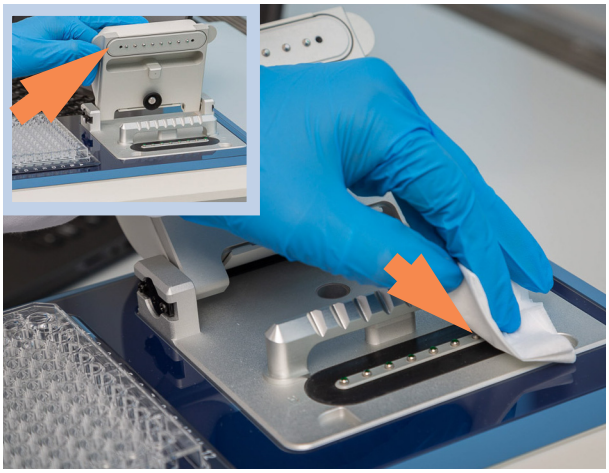
To measure a micro-volume sample

NOTICE

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



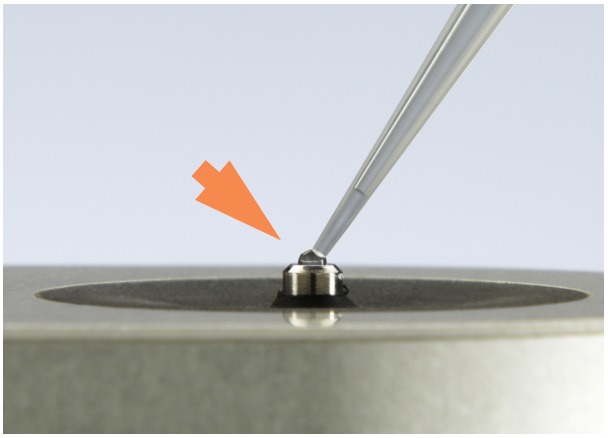
1. From the Home screen, select an application from one of the application categories, such as **UV-Vis**, or **Custom** Methods.



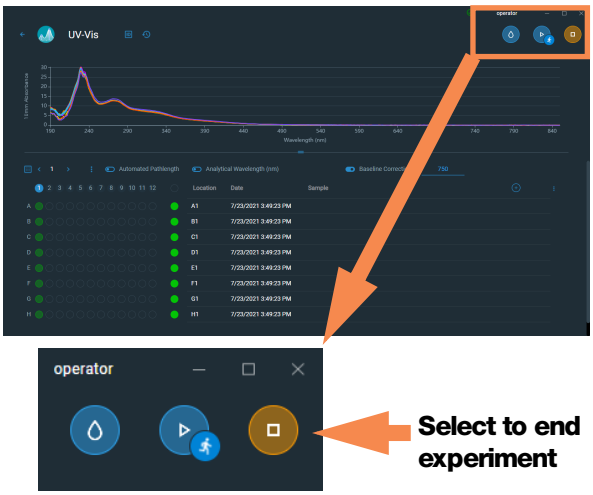
2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.

3. Measure a blank:
   - Pipette 1–2 µL blanking solution onto the lower pedestal and quickly lower the arm
   - Click **Blank** and wait for the measurement to complete

     Tip: If Auto-Blank is On, blank measurement starts automatically after you lower the arm.
   - Lift the arm and clean all pedestals with a new laboratory wipe



4. Measure the first sample:
   - Pipette 1-2 µL sample solution onto the pedestal and quickly lower the arm (see Recommended Sample Volumes for more information)
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and click **Measure**
   - When the sample measurement is completed, the spectra and reported values are displayed.



5. To measure another sample:
   - Lift the arm
   - Clean all pedestals with new wipe
   - Load the next sample(s) and quickly lower the arm
   - Start the sample measurement
   - Wait for the measurement to complete

   The new spectrum replaces the previous one on the spectral display and the new reported values replace the previous ones in the table.

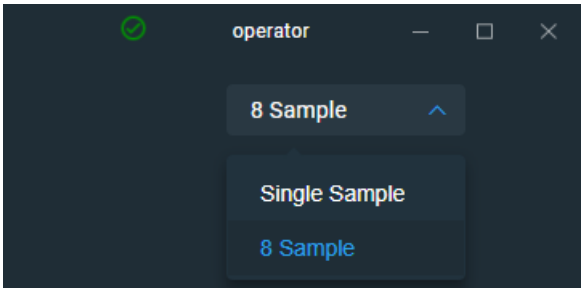
6. When you are finished measuring samples:
   - Select the End Experiment icon (see previous image)
   - Enter an experiment name or leave the default experiment name
   - Select Save
   - Lift the arm and clean all pedestals with a new wipe

     If finished with the instrument for the day, clean the pedestals with DI H2O (see Clean pedestals between users)

Acquired data are automatically saved in an experiment with the entered name. In the default configuration, experiments are stored in a database according to acquisition date, experiment name, application used and any assigned labels (see Manage identifiers).

Sample Modes

The NanoDrop Eight can be used in either Single sample or an Eight sample mode. Select the desired mode from the drop-down menu on the Home screen. In Single Sample mode, measurements will only be taken using Channel A.



Module startup

Instrument self-test and preparation begins upon selection of an application after first launching the software. The message 'Please wait - Initializing Spectrometer' will appear. When this message disappears, the instrument will be ready for use and you can prepare blanks. All data taken will automatically be logged in the appropriate archive file.

Sample Plate Map

When running in Eight Sample mode, a plate map will display in the sample area. This on-screen sample plate will populate with Sample ID information from an imported sample ID file. You can also manually enter Sample ID or other identifying information. The sample status color code displayed on the plate map is determined by plate configuration at set up. See “Sample Position Illuminator” on page 174 for details.

Prepare Samples and Blanks

Before making a sample measurement, a blank must be measured and stored. All eight channels are blanked with each blanking command when using the 8 sample mode. Only channel A is blanked for the single position mode.

Note: When using the 8 Sample mode, the software initiates each blank and measurement cycle on the first position to be read. The user will, therefore, hear one less position increment than expected. After making an initial blank measurement, a straight line will appear on the individual graphs. Subsequent blanks will clear any sample spectrum and again display straight baselines.

Preparing Samples

- Isolate and purify samples before measuring them with the instrument. Commercial sample isolation kits are available for these purposes, or use an in-house protocol. After purification, analyte of interest is typically dissolved in aqueous buffer solution before it is measured.

  Tip: Any molecule that absorbs light at analysis wavelength will contribute to total absorbance value used to calculate sample concentration.

- Ensure final analyte concentration is within instrument's absorbance detection limits.
- For micro-volume measurements, gently (but thoroughly) vortex each sample before taking a measurement.

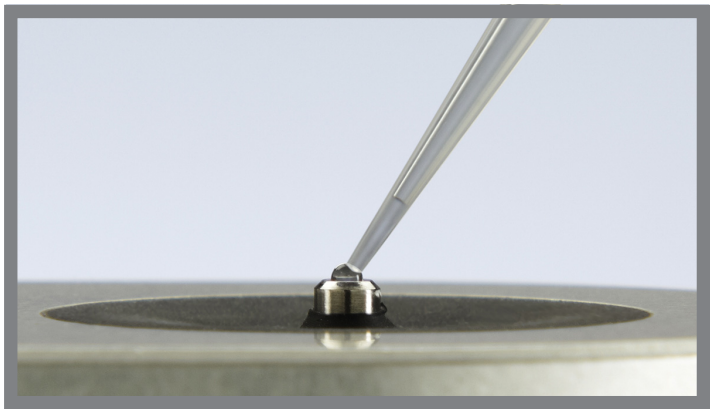
Avoid introducing bubbles when mixing and pipetting.

Choosing and Measuring a Blank

The buffer used to resuspend a sample analyte can contribute absorbance. Blanking minimizes any absorbance contribution due to the buffer components from the sample measurement. The resulting sample spectrum represents the absorbance of only the analyte of interest.

For best results:

- For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution. For details, see "To measure samples" in the application used.
- Measure new blank before each set of samples. It is not necessary to blank the instrument before each sample measurement unless the samples are dissolved in different buffer solutions.
- It is recommended to measure a new blank every 30 minutes.



- Run a blanking cycle to assess the suitability of your blanking solution before using it to perform sample measurements. For a quick demonstration, watch the multimedia training Evaluating a Blanking Solution for Suitability.

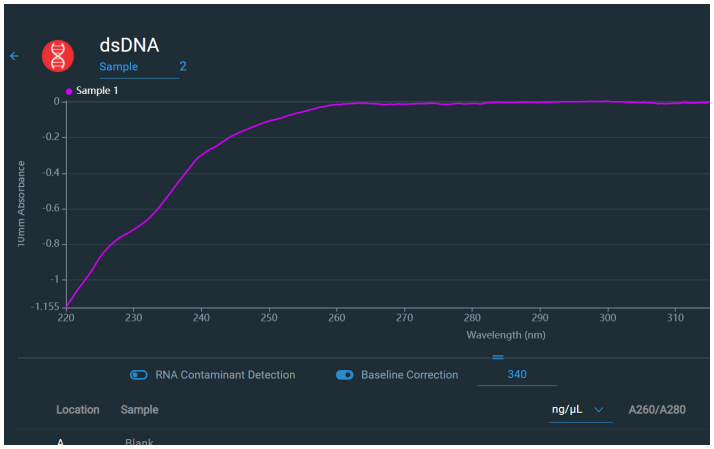
  The resulting spectrum should vary no more than 0.04 A (10 mm equivalent) across the spectrum, especially at the analysis wavelength as in the example at the right.

  If the resulting spectrum is greater than 0.04 A around the analysis wavelength, that buffer solution may interfere with the sample analyses, especially for low concentration samples. See below for details.

Good blanking buffer (measured abs < 0.04)

Problems associated with blanking

- Residual sample was left on pedestal before blank measurement was performed. (Resulting sample spectra may exhibit negative absorbance values, indicating blank had more absorbance than sample in that region of spectrum.)
- Blank measurement exhibits higher absorbance than unknown sample at analysis wavelength. (If buffer used as blank differs in composition from that used to resuspend sample, measurement results will be incorrect.)
- Sample was inadvertently used to blank instrument. (Resulting sample spectra may exhibit negative absorbance values or, in some cases, resemble a mirror image of a typical pure nucleic acid or protein spectrum.)



Solution containing salt used to blank instrument results in “mirror image” spectrum

Solutions for blanking problems

- Thoroughly clean and/or recondition both pedestals and then:
  - rerun blanking cycle, or
  - measure new blank using new aliquot of appropriate buffer solution, then measure new aliquot of unknown sample
- For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution. For details, see “To measure samples” in the application used.

Run a Blanking Cycle

Run a blanking cycle to verify the following:

- instrument is operating normally (with flat baseline)
- pedestals are clean (i.e., no dried-down sample material on pedestals)
- absorbance contribution of buffer solution you plan to use for sample analyses

Supplies needed

- lint-free laboratory wipes
- calibrated precision pipettor (0–2 µL)
- buffer solution for evaluation

To run a blanking cycle

For quick demonstration, watch multimedia training Evaluating a Blanking Solution for Suitability.

NOTICE

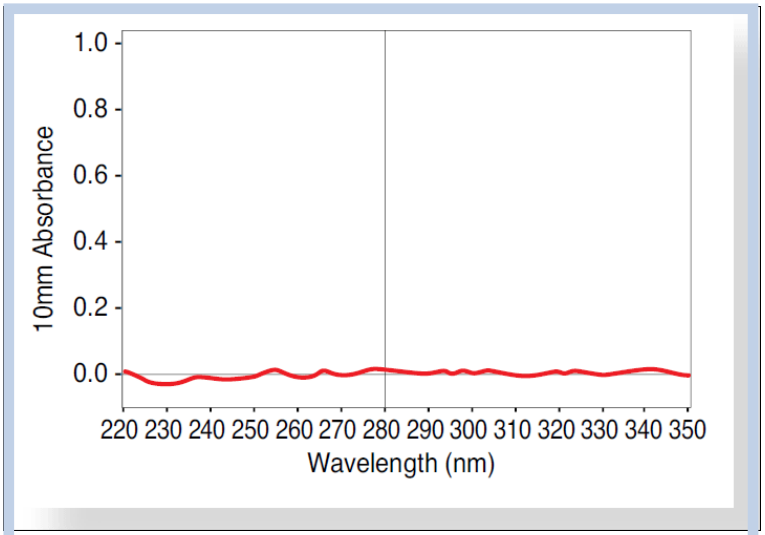
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

1. From the Home screen, select an application name.
2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.
3. Measure a water blank:
   - Pipette exactly 1 µL deionized water (DI H_2O) onto the lower pedestal and lower the arm.
   - Select **Blank** and wait for the measurement to complete.
   - Lift the arm and clean all pedestals with new laboratory wipe.
4. Measure the buffer solution:
   - Pipette 1-2 µL buffer solution onto the pedestal and lower the arm.
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and select **Measure**
   - Wait for measurement to complete.

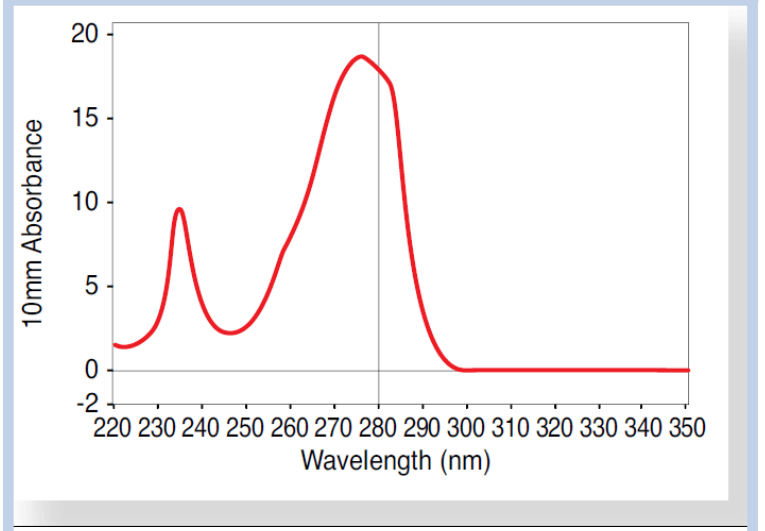
   The resulting spectrum should vary no more than 0.04 A from the baseline at the analysis wavelength.

   If your spectrum does not meet these criteria, repeat steps 2–4.

   If spectrum is still outside specifications, see Solutions for Blanking Problems.
5. When you are finished with the blanking cycle, select **End Experiment**.
6. Lift the arm and clean all pedestals with a new wipe.



Example spectrum of buffer suitable for Protein A280 protein quantification

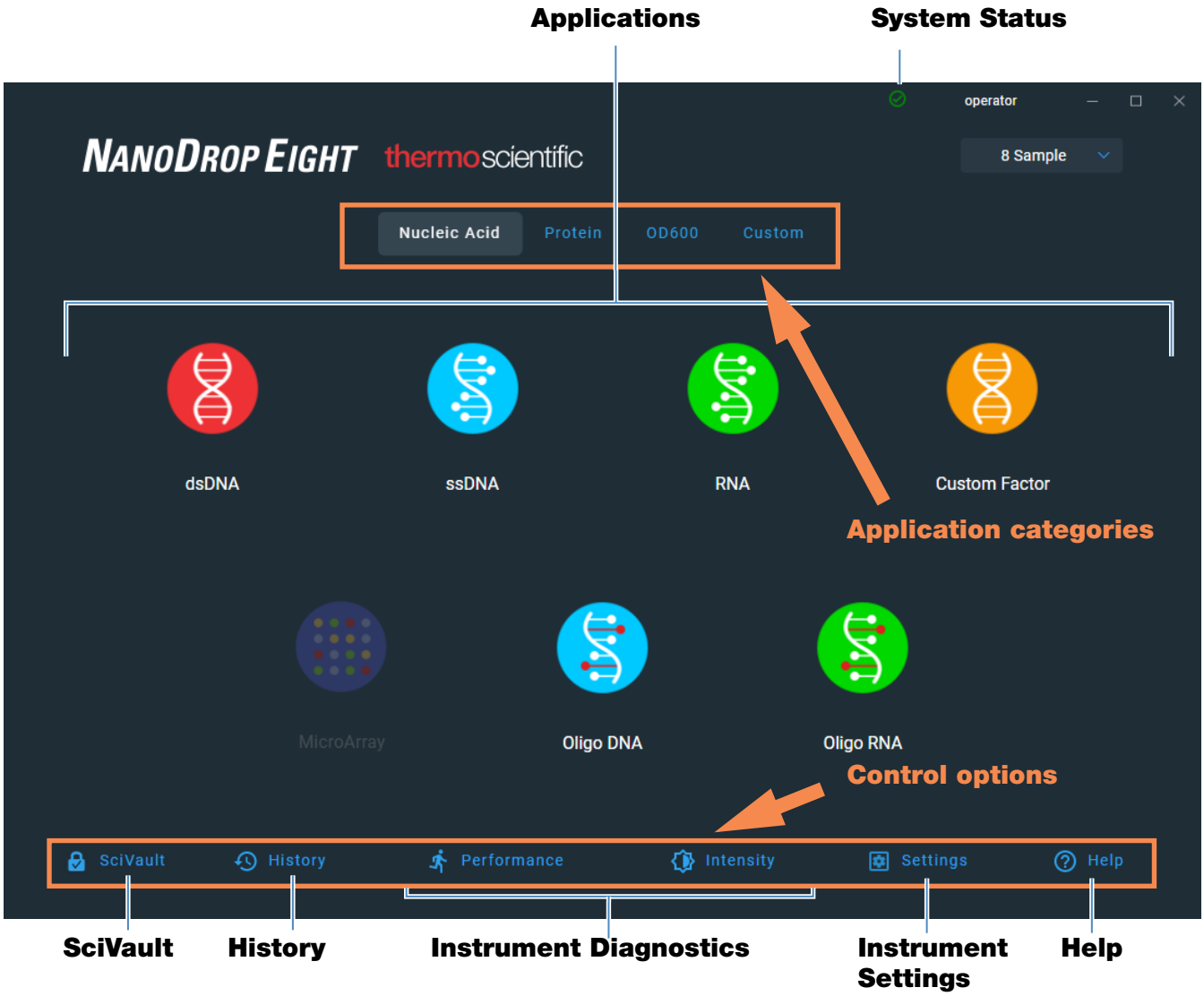


Example spectrum of buffer unsuitable for Protein A280 protein quantification

Basic Instrument Operations

NanoDrop Eight Home Screen

These operations are available from the NanoDrop Eight Home screen.

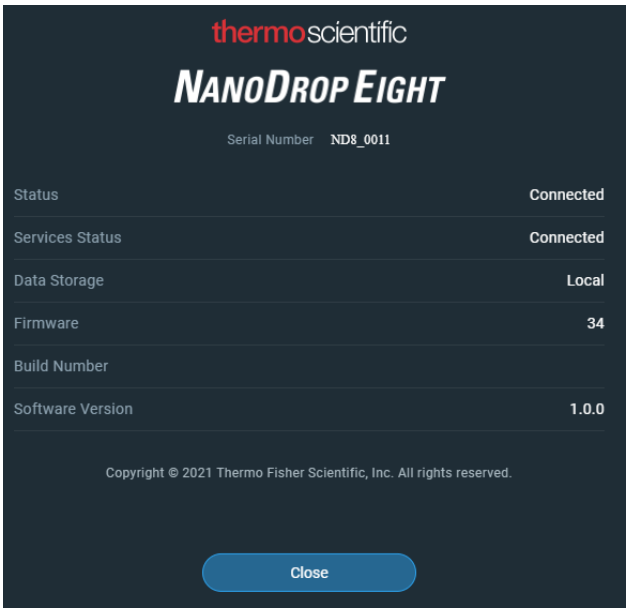


Applications

The NanoDrop Eight software offers several configurable applications, which gives users full control of the measurement. See"Custom Applications" on page 129 for detailed information about each available application.

System Status

Select the icon on the Home screen to open the system status box.



The available information is described below.

Serial number	Instrument serial number
Status	Current status of the instrument
Data storage	Indicates location of database set where instrument is currently storing data.
Firmware	Version of instrument firmware installed
Build Number	Current Software build
Software Version	Version of instrument operating software installed

Control options

History: View data stored locally. Filter by date or application.

Performance: Performance verification process using PV-1 solution. See "Performance Verification" on page 212

Intensity: Run an intensity check for the pedestal. See "Intensity Check" on page 210.

Settings:	Set security server location and path if desired.
Help:	View help

History

Select History on the Home screen to view any data acquired earlier today, last week, last month, last six months, last year or in a specific date range. See "View History" on page 176 for more information about the History feature.

Instrument Settings

Select Settings on the Home screen to access instrument settings for software updates, Protein Editor, and more. See "Instrument Settings" on page 195 for detailed information about all available instrument settings.

Instrument Diagnostics

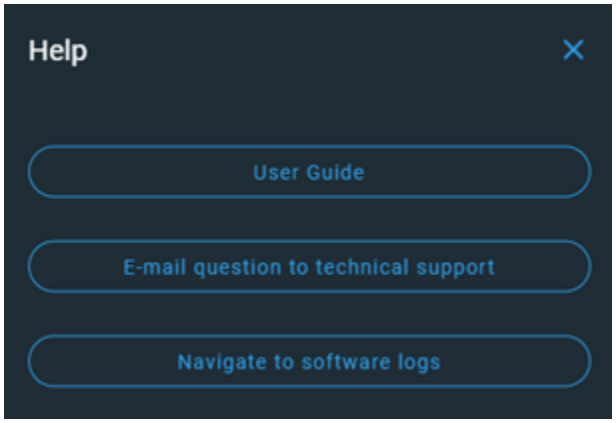
Instrument diagnostics (Performance and Intensity check) should be run periodically according to the recommended maintenance schedule. See "Instrument Diagnostics" on page 209 for information about how to run the available instrument diagnostics.

SciVault Software

The Thermo Scientific™ SciVault™ software is available as an optional add-on. This companion software allows users to operate their NanoDrop Eight instrument in a manner that complies with US FDA 21 CFR Part 11. When purchased, the SciVault software is shipped to you on a USB stick and integrates directly into the NanoDrop Eight software user interface. For more information, visit thermofisher.com/nanodrop.

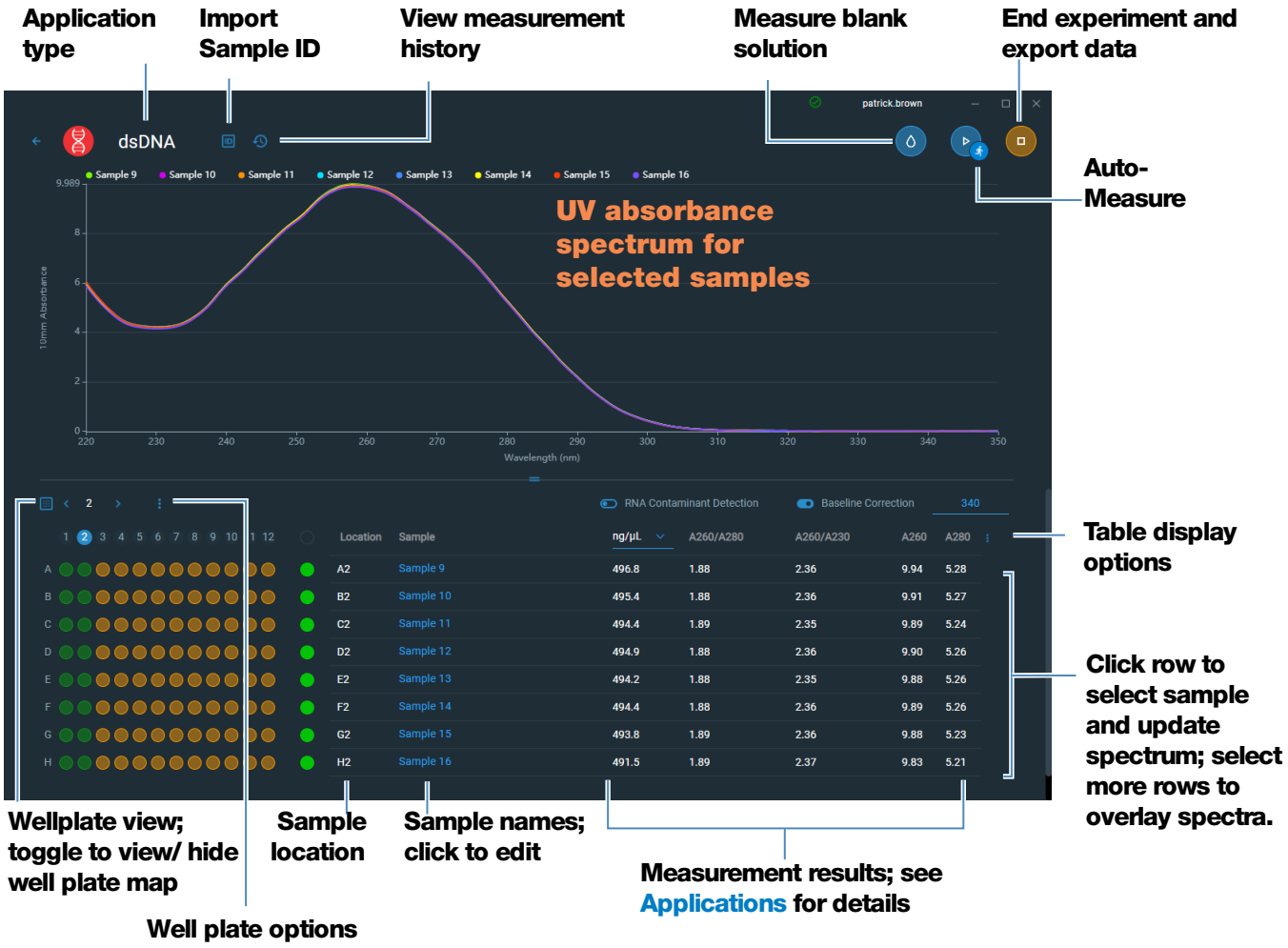
Help

Select Help to launch the user guide, email a question to tech support, or find the software logs.



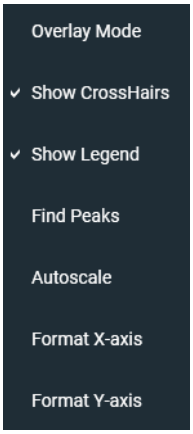
NanoDrop Eight Measurement Screens

These operations are available from any measurement screen within an Application.



Measurement Screen Display Options

When performing measurements, right-click the graph on the measurement screen to bring up the following display options:



Display multiple samples in overlay.

Hover spectrum to see plot data

Display graph legend

Calculate peaks for specified range

Scale axes to fit spectra measurement

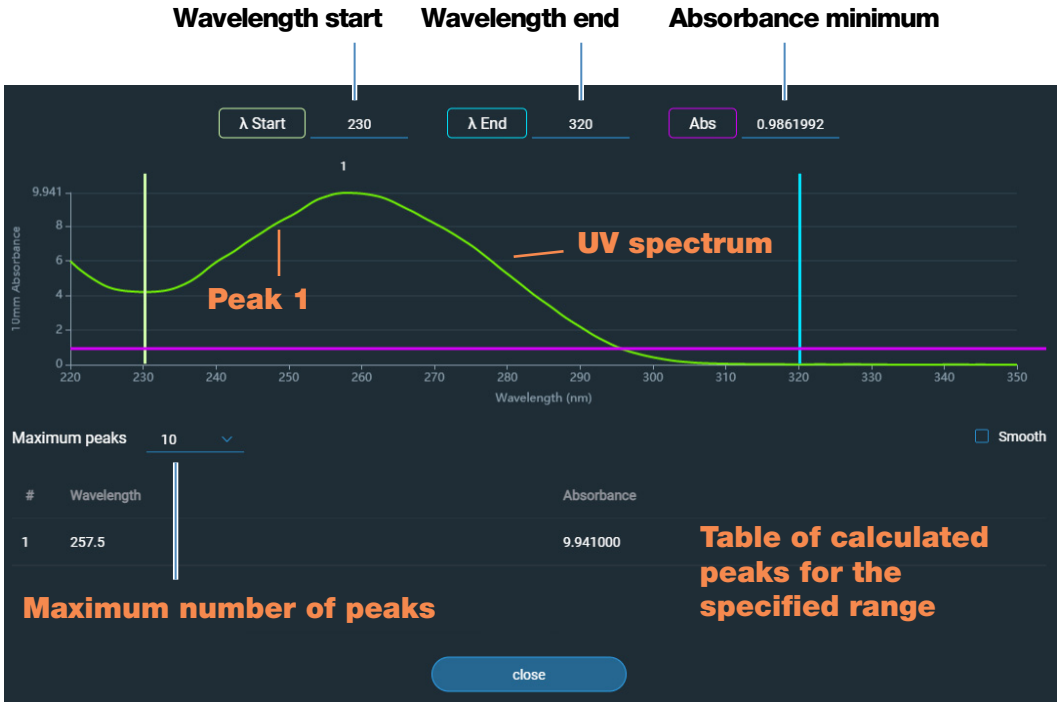
Select to manually enter x-axis range

Select to manually enter y-axis range

Display options- Right click graph to view

Find Peaks

Select **Find Peaks** to view calculated peaks for specified range. You can enter the range by dragging the color-coded limit lines, or enter values into the fields at the top of the spectrum. Found peaks for the defined range are listed in the table below the spectrum.



Sample Name

Click the Sample Name field in any measurement screen to edit the sample name.

In Single Sample mode, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name.

In 8 Sample mode, each sample is automatically labeled by the sample location. Add sample names by clicking the sample name field, or by importing Sample ID file.

Note If you edit the sample base name during an experiment when Auto-Naming is selected, the assigned sample ID numbers restart.

Edit sample base name

After you measure a blank and before the first sample is measured:

- click **Sample Name** field
- enter new base name
- press **Enter** key

Edit sample name

- from Home screen, click 

to open History
- select experiment
- select name field of sample
- enter new sample name
- press **Enter** key

Measurement Results

The types of results that appear in the measurement screens depend on the selected application. For details, see the reported results section of that application in this guide:

Applications > [application group] > Measure [application name] > Reported Results

Absorbance Spectrum

For each measured sample, each application shows the UV or UV-Visible absorbance spectrum and a summary of the results. The vertical axis shows absorbance in absorbance units (A). The horizontal axis shows wavelength in nm. Here is an example for a UV-Vis method.

Sample Pathlength

All applications display the sample pathlength along the spectrum's vertical axis. Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

Measurement Alerts

The Acclaro Sample Intelligence technology built into the NanoDrop Eight instrument provides important features to help you assess sample integrity. click a Sample Intelligence icon in the software to view its associated information.

contaminant analysis is available to help qualify a sample before use in downstream applications

on-demand technical support is available for measurements that are atypical or very low concentrations

Blank Button

Select **Blank** to measure a blank for the selected experiment.

A blank must be measured before each group of similar samples. The blank solution is typically the pure buffer that was used to resuspend the sample. For more information, see Choosing and Measuring a Blank.

Measure Button

Select **Measure** to measure a sample for the selected experiment.

Samples must be properly isolated and prepared before they can be measured with the instrument and the concentration must be within the instrument's absorbance detection limits. For more information, see Preparing Samples, Measure a Micro-Volume Sample, and Absorbance Detection Limits.

Note The **Measure** button is enabled after a valid blank measurement is completed.

Auto-Measure and Auto-Blank Options

Speed up sample analysis with the NanoDrop Eight Auto-Measure and Auto-Blank features, which cause the instrument to start the measurement immediately after you lower the instrument arm. These options eliminate the need for repetitive Measure or Blank operations for large batches of samples.

Note Auto-Measure and Auto-Blank are available for micro-volume measurements only.

Auto-Measure

To select or deselect Auto-Measure, from any sample measurement screen, click the **On** or **Off** button at the right of the Measure button.

Auto-Blank

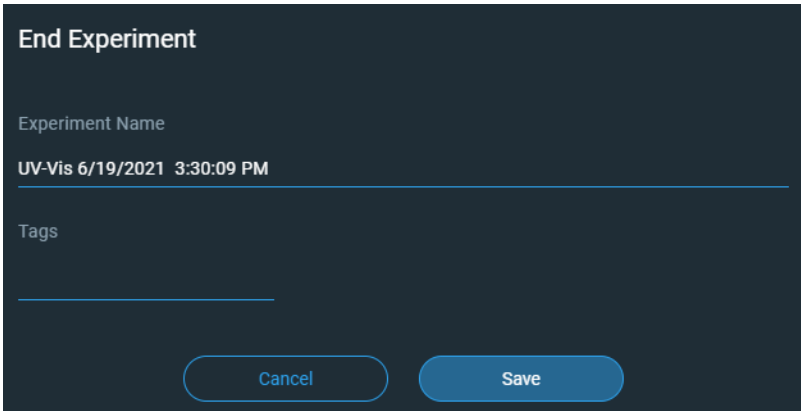
To select or deselect Auto-Blank, from any blank measurement screen, click the **On** or **Off** button at the right of the Blank button.

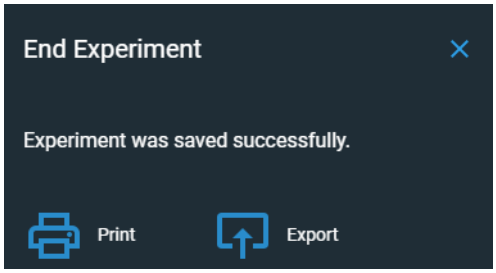
End Experiment Button

Select **End Experiment**  when you are ready to name and save your experiment, add a label to help you locate the experiment later or export the data. Depending on the administrative settings, you may be prompted to sign the experiment upon ending the experiment.

Note The **End Experiment** button is enabled after the first sample measurement is completed.

After you select End Experiment, the End Experiment dialog box is displayed:





Click Save and you will have the option to Print or Export the experiment data.

Available options:

Experiment Name	Enter a name for this group of measurements. The measurement results are saved in the selected database location using the entered experiment name.
Tags	Enter a descriptive label to help you find this experiment later or to associate it with another experiment (see Manage identifiers on the instrument for details).

Export	Select an available location for exporting the measurements in this experiment. Experiments can be exported to a USB device connected to the PC, any USB port, or to a network location. Available export file formats: • comma-separated values spreadsheet (.csv) file • tab-separated values spreadsheet (.tsv) file • NanoDrop Eight software (.n8db) file The filename is the entered experiment name (see above). The file is stored in a folder named "NanodropEight" followed by the instrument serial number. (Use System Status to view your instrument serial number.)
Cancel (Return To Experiment)	Close the End Experiment box and display the results for the most recent measurement. From there you can add measurements to the current experiment and save it later.
Print button	Print measurement results for current experiment
Save (End Experiment)	End the experiment and save the measurement results using the entered experiment name. The experiment is saved in the selected database location.

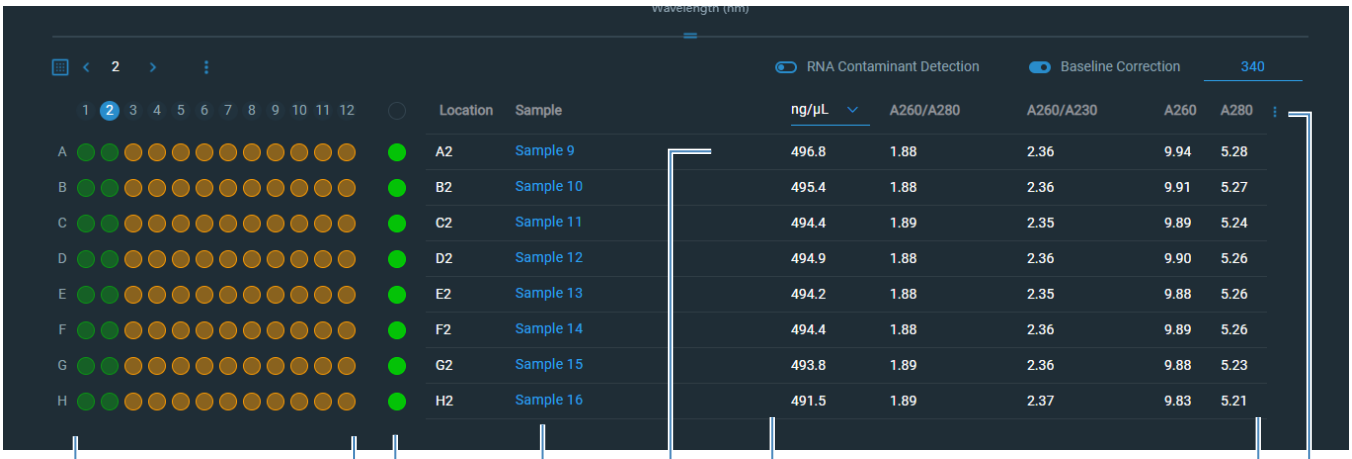
Sample Details

Sample details are shown in the **sample row** in any measurement screen or History. This information includes all available measurement results and associated details for the selected sample. To add or remove results shown in the sample row, click the menu in the upper right corner of the data table. Information about the measured values displayed in Sample Details is provided in this Help system, under the application used to acquire the data.

Note You can also edit the sample name by clicking in the name field of the sample.

Data Table

The data table for the current experiment is shown below the spectrum graph. The data table contains the measurement results for all samples in the experiment. The image below highlights the available features.



Well plate map

Sample name; click to edit

Measurement results; see Applications for details

Menu of options; click to open

Active channel indicator; click to activate or inactivate sample locations to add to measurement

click row to select sample

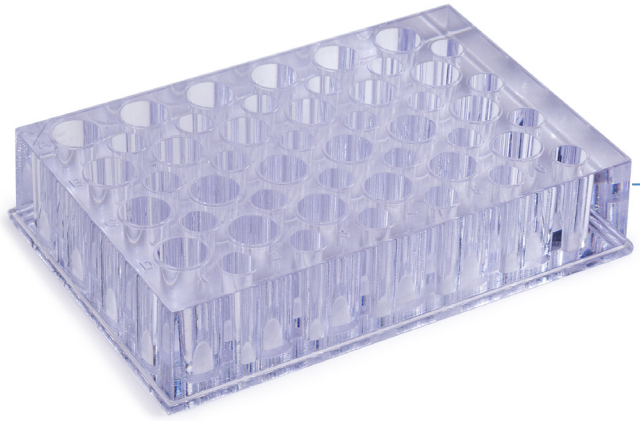
Data Table is in the bottom half of the measurement screen

Sample Position Illuminator

The NanoDrop Eight Sample Position Illuminator and accompanying software user interface are designed to help users keep track of which samples have already been measured and which samples should be measured next. There are three different templates designed for different sample holding formats. You can choose between 96-well plate, 1.5 mL tubes, and 0.5 mL tubes by clicking the 3-dot menu above the plate matrix user interface in the bottom left of the measurement acquisition screen.

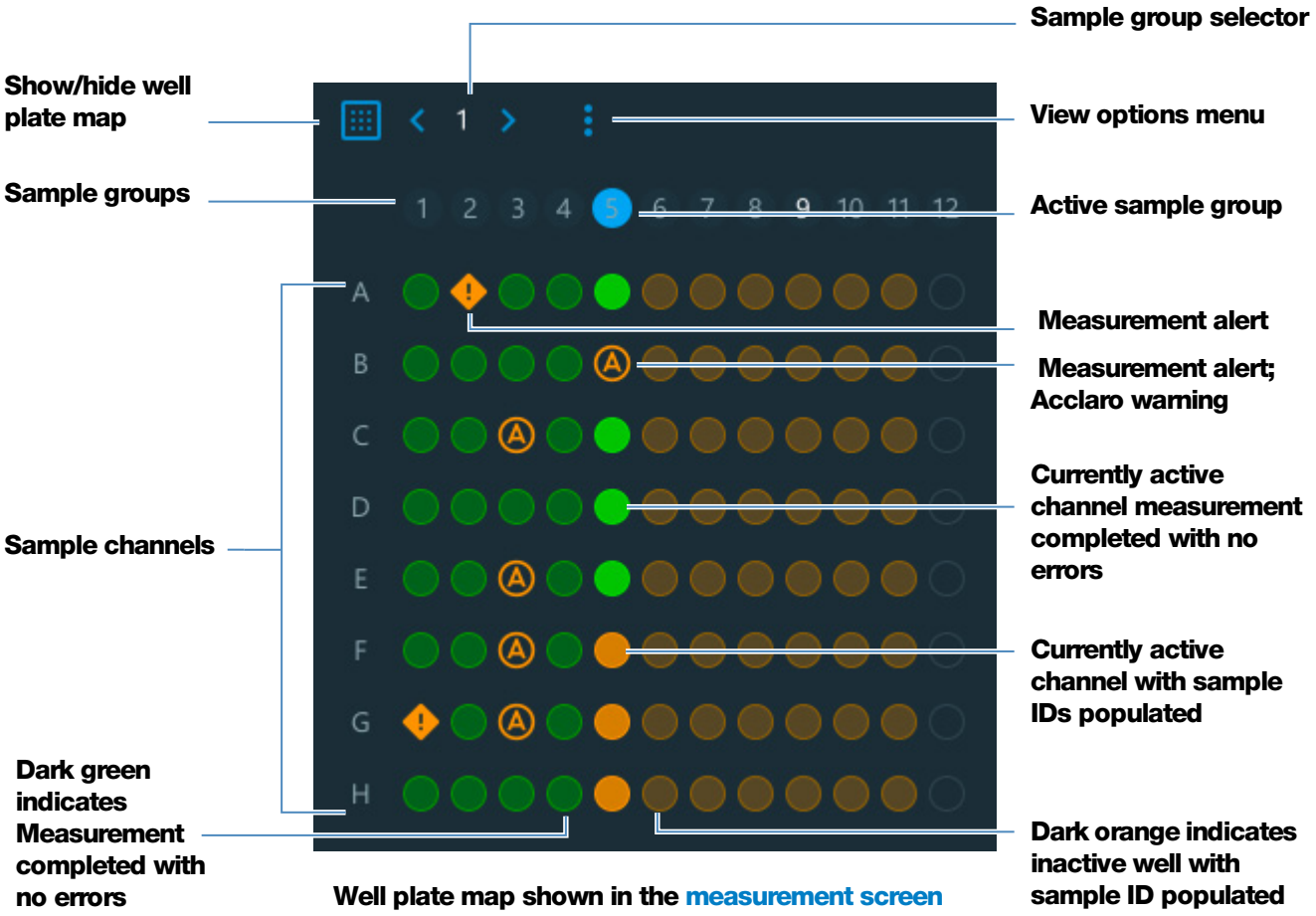
- 96-well plate: allows users to keep track of sample measurements for samples stored in a standard 96-well microtiter plate
- 1.5 mL tubes: allows users to keep track of samples measurements for samples stored in the 1.5 mL-sized spaces in the NanoDrop Eight tube rack accessory
- 0.5 mL tubes: allows users to keep track of samples measurements for samples stored in the 0.5 mL-sized spaces in the NanoDrop Eight tube rack accessory

NOTE: This feature works most efficiently when a user imports sample names from a file.



NanoDrop Eight tube rack accessory

The wells/tubes which should be measured next are filled with bright orange in the software user interface. They correspond to the wells/tubes illuminated by the green LEDs on the top of the instrument. Wells/tubes that have sample IDs pre-populated will be filled with light orange. Wells/tubes that have already been measured will either be filled bright green (indicating no sample alerts) or with an A in a circle (indicating an Acclaro alert is present for that sample).



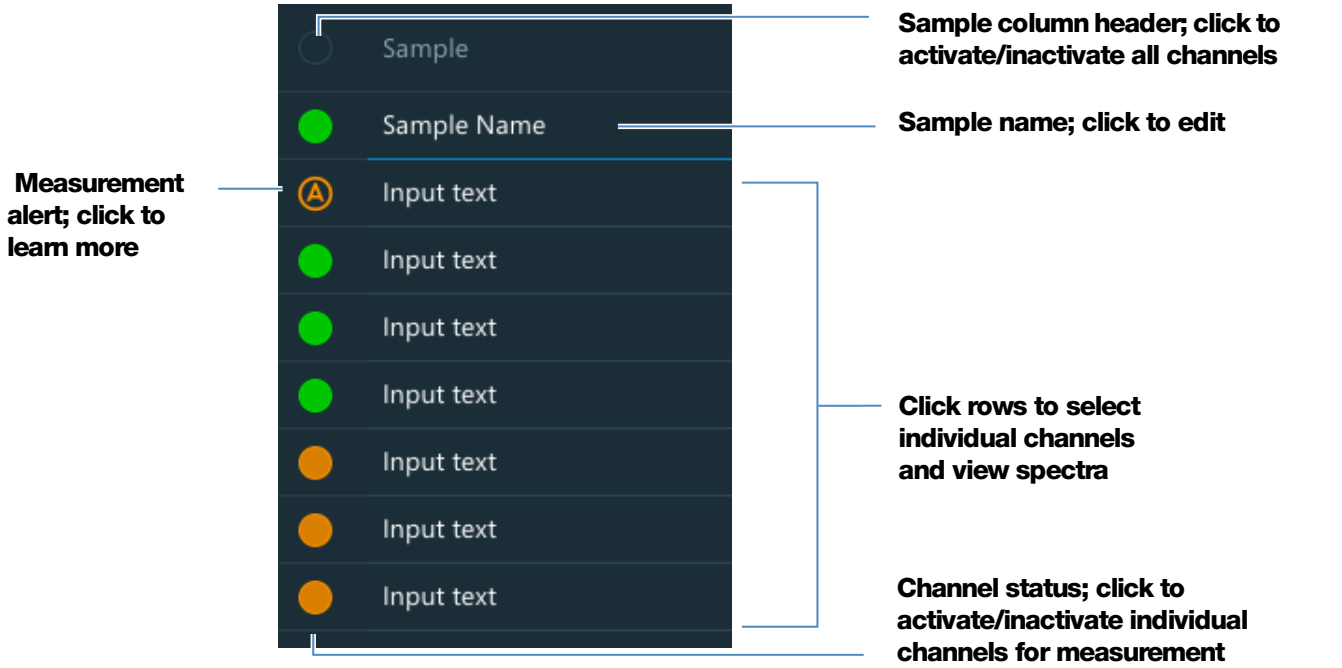
Well plate map shown in the measurement screen

After a set of measurements is complete, users can manually advance the sample names and well illumination by clicking the < and > arrows above the well plate map in the bottom left. This will prepare the instrument software for the next set of measurements. Alternatively, users can click the 3-dot menu above the well plate map and enable Auto Advance. Auto Advance sets the software to automatically advance the columns a few seconds after the last measurement is made.

The entire well plate map interface can be hidden by clicking on the plate icon .

Manual Sample ID Entry

Users can manually enter sample names using a keyboard or barcode scanner. When sample names are entered manually, the user needs to activate each channel they wish to measure. To activate/inactive all channels at once, click the circle in the column header area next to Sample. Alternatively, users can activate/inactivate individual channels by clicking the circles within each row.



Active channel indicator of the measurement screen

View History

Whether you collect one sample or many in a row, after you choose End Experiment, the acquired data are automatically saved in an experiment with an experiment name. In the default configuration, experiments are stored in the NanoDrop Eight database according to acquisition date, experiment name, application used and any assigned labels.

Use the History feature to open the database in order to view acquired spectra and associated data from any experiment at any time.

Open instrument database of measurement results

– to open NanoDrop Eight database, from the Home screen, select History.

History Menu

Available options:

Home	Return to NanoDrop Eight Home screen
Import	Import data from a USB flash drive or folder on your computer or network hard drive
Export	Export the experiment

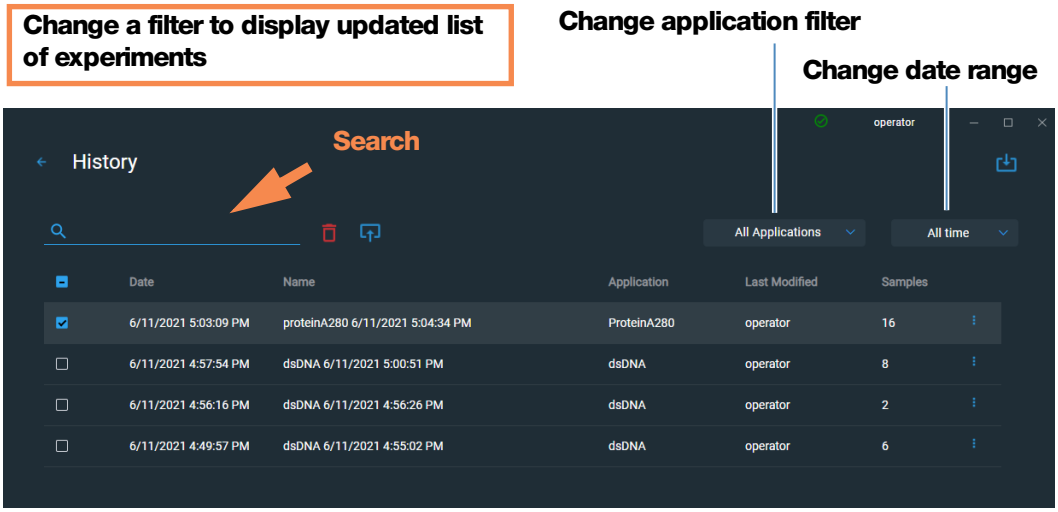
Measurement Menu

When viewing measurement data from History, Click the menu in the upper right of the measurement screen to access available menu options.

Export	Export the experiment
Print	Print selected measurement results
Tags	Manage experiment tags
Details	Experiment details such as Name, Application type, Creation date, and number of measurements

Search Experiment Database

Use the search feature in History to search the selected database for an experiment or to change the time range or other search filters. The database is filtered using the current settings in the Search box. Filters include time range, application type and any user-defined labels (see Manage Identifiers for information about adding and deleting labels). Here is an example:

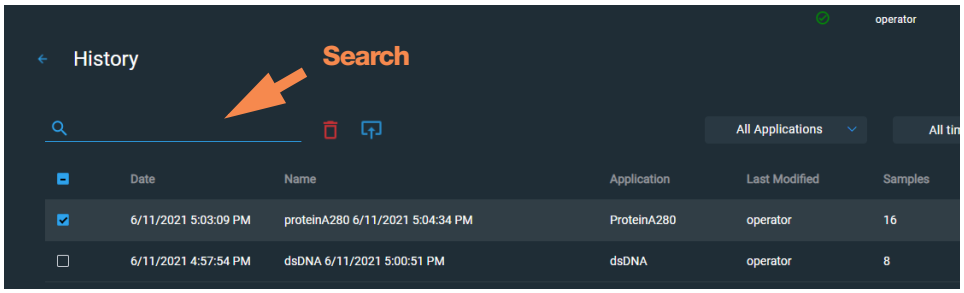


Export Selected Experiments

Use **Select** in History to select experiments to be exported.

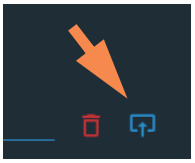
Export selected experiments

– Open History. You can filter or use the Search feature to find experiment

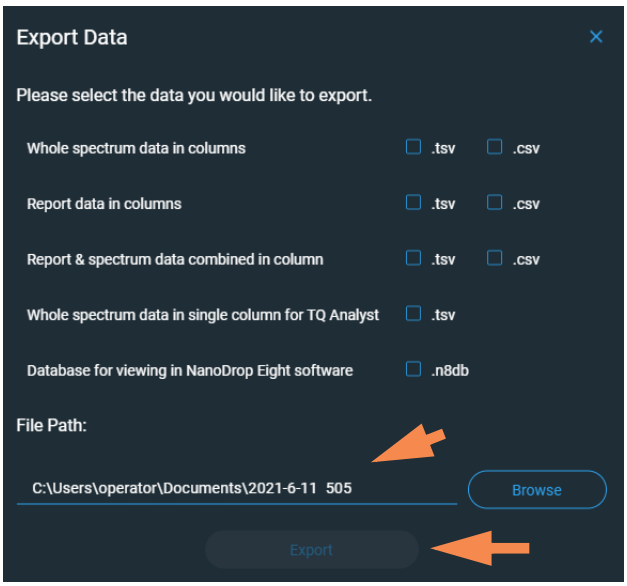


– click the checkbox to select row or rows to be exported

– click **Export**



– select one or more formats to export to (see “Export Selected Experiments”)



– enter or browse to an available export location USB drive, PC storage, or a network location) and select **Export**

– after “Export Success” message, select **OK**

Delete Selected Experiments

Select experiments to be deleted.

Delete selected experiments

– Filter experiments as needed, use Search feature to find desired experiment

– Click the checkbox of the **row** or rows to select one or more experiments to delete (click again to deselect an experiment)

– Click **Delete** and **OK**

Note Deleted data cannot be recovered.

Open Experiment and View Associated Data

Use History to locate and open any experiment to see the measurement data it contains.

Open an experiment

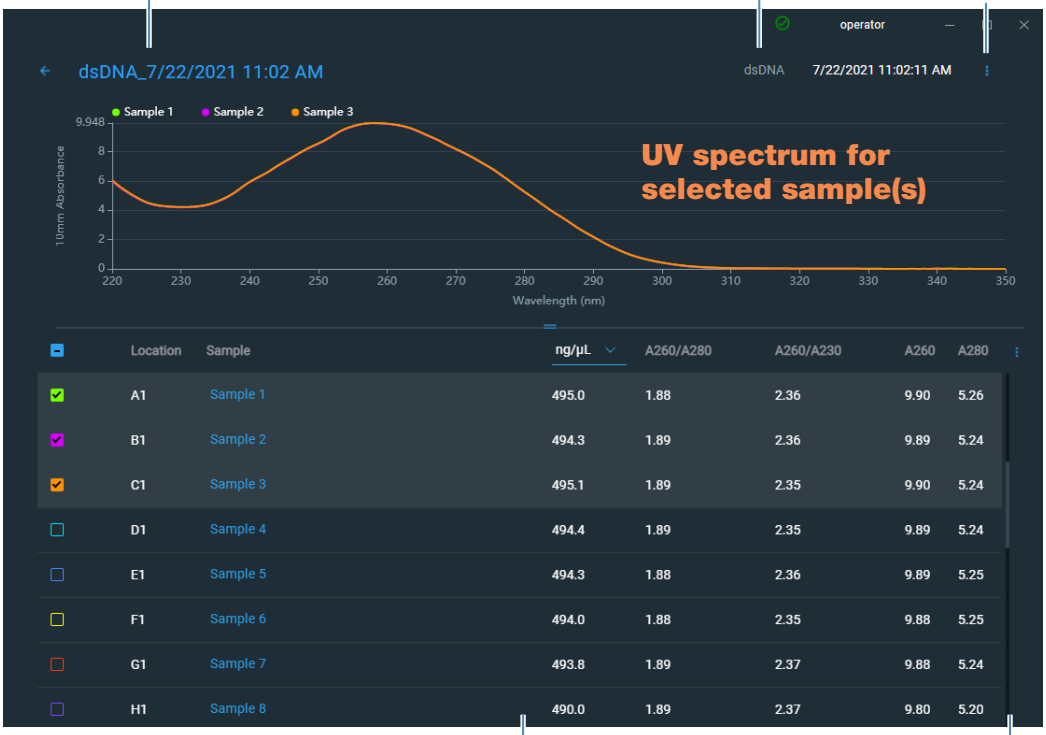
- In History, if you don't see the experiment you want to open, you can use the Search feature to find the desired experiment
- click the **experiment name** to open the experiment

The History provides measurement data as spectral data and data tables, similar to what you see after you complete a measurement.

Note The data shown are dependent upon the application used to measure the samples (nucleic acids in these examples). For more information, see the application details.

Spectral data—

After you open an experiment, the software shows the UV or UV-visible absorbance spectrum and associated data for the selected experiment, much like it appears during a measurement. The image below describes the available features.



Data Table—

The lower area of the measurement history screen (shown above) shows the data table for the current experiment. The data table contains the measurement results for all samples in the experiment. The image below describes the available features.

Menu

Select the menu in the upper right corner from any Spectral Data or Data Table screen to see the available menu options.

Home	Return to NanoDrop Eight Home screen
Manage Identifiers	Add or delete labels for selected experiment to make it easier to find (see Manage identifiers on the instrument)
Export	Export selected experiments
Print	Print plot or data table for selected measurement results; if no results are selected, prints all results in data table
Settings	View or change instrument settings

NanoDrop Eight General Operations

These operations are available from any measurement screen or from the History.

Manage Identifiers

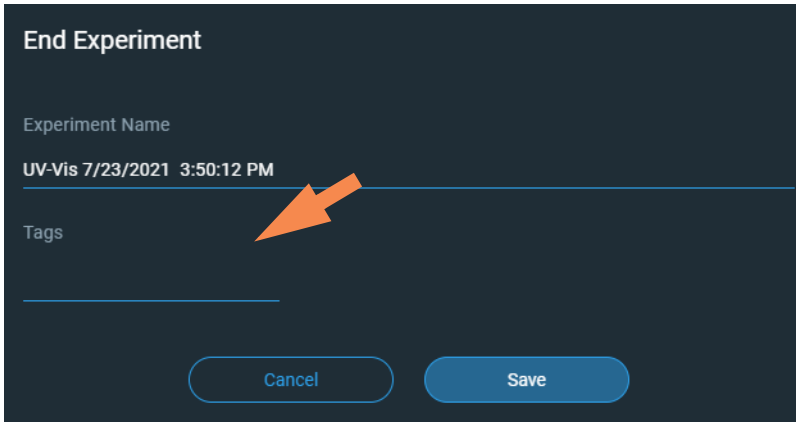
You can add one or more “tags” (i.e., labels or metadata tags) to an experiment to make the experiment easier to find.

Use the History to add labels to experiments, assign existing labels, view assigned labels and remove or delete labels on the instrument. You can filter the list of experiments in the History based on one or more user-defined labels.

Label new experiment when you save it

– after the last sample has been measured, select  End Experiment.

– in End Experiment box, enter user defined labels in the **Tags** field



– click **Save**

Label experiment in History

– from the Home screen, select **History**

– Click the menu of the experiment row and select **Tags**

– in Manage Tags box, enter a tag as an **Identifier**

– Click Save to add the tag. Add additional tags as needed.

– Click **OK**

Remove a label

– from Home screen, select **History**

– Click the menu of the experiment row and select **Tags**

– in Manage Tags box, click the **X** for any tag you wish to delete

– Click **OK**

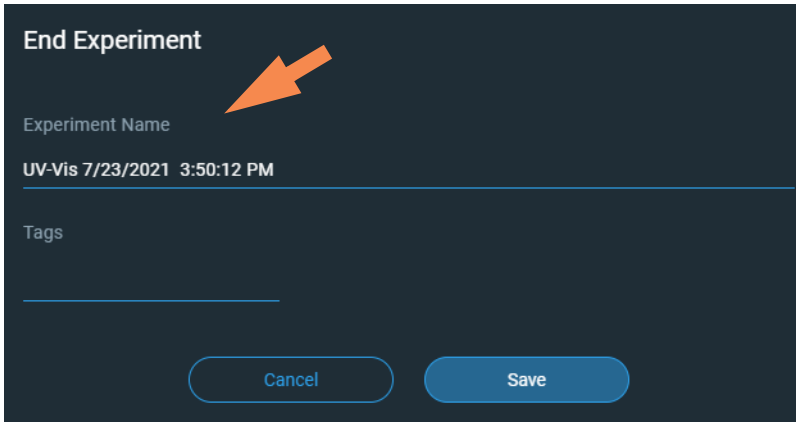
Edit Experiment Name

You can edit the experiment name when you save the experiment or afterwards from the History.

Edit experiment name at end of experiment

– when finished measuring samples, select End Experiment

– enter a name for this group of measurements in the Experiment Name box



– click **Save**

Edit experiment name from History

– from Home screen, open History
– use Search feature to find experiment
– Double click experiment to open experiment
– Click the **Experiment Name** field
– enter new experiment name
– the new name is saved automatically

Export Selected Experiments

You can export measurement data when you save the experiment or afterwards from the History

Note Data exported during a save are still saved to a database (local or remote, depending on the Data Storage setting.

Measurement data can be exported in three formats:

- as comma-separated values (.csv) files containing:
  – whole spectrum data in columns
  – report data in columns
  – both report and spectrum data combined in columns
- as tab-separated values (.tsv) files containing:
  – whole spectrum data in columns
  – report data in columns
  – both report and spectrum data combined in columns

  - whole spectrum data in single column for TQ Analyst

- as NanoDrop Eight software (.n8db) files containing spectra and measurement results for each exported experiment

Use any spreadsheet or word processing application to open a CSV or TSV file. Here is an example of several sample measurement results in CSV format:

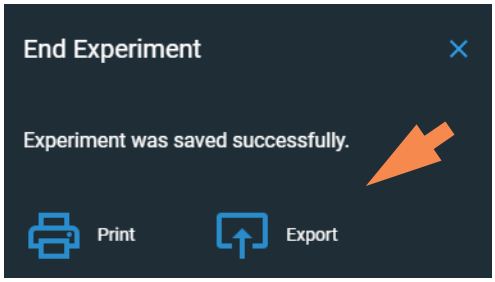
	A	B	C	D	E	F	G	H	I	J
1	Application:	dsDNA								
2	Serial Number:	ND8_0014								
3	User Name:	p.brown								
4	Sample Id	Sample Name	Date & Time	Location	ng/µL	A260/A280	A260/A230	A260	A280	
5	ef545555-6982-4cf1-	Sample 1	7/22/2021 11:03	A1	494.9728	1.8826	2.3554	9.8995	5.2584	
6	175af623-c7b2-4e94	Sample 2	7/22/2021 11:03	B1	494.3488	1.8854	2.3578	9.887	5.2441	
7	ef763938-31ba-4eb9	Sample 3	7/22/2021 11:03	C1	495.1052	1.8885	2.3474	9.9021	5.2432	
8	16031e24-e1d6-462	Sample 4	7/22/2021 11:03	D1	494.3507	1.8852	2.349	9.887	5.2446	
9	a08fc6f9-bd2e-4f28-	Sample 5	7/22/2021 11:03	E1	494.3455	1.8823	2.3554	9.8869	5.2525	
10	0c88c4ce-0fdd-489e	Sample 6	7/22/2021 11:03	F1	493.9864	1.8827	2.3517	9.8797	5.2476	
11	69e3aac4-50da-4244	Sample 7	7/22/2021 11:03	G1	493.788	1.8861	2.3715	9.8758	5.2361	

Note The types of data exported are dependent upon the application used to measure the samples (nucleic acids in this example). For more information, see the application details.

Data can be exported to any file path connected to the PC or to a network location. If you select multiple experiments for export, each exported experiment has a corresponding file. The filenames are the same as the experiment names. The files are stored in a folder named “NanodropEight” followed by the instrument serial number. (Use System Status to view your instrument serial number.)

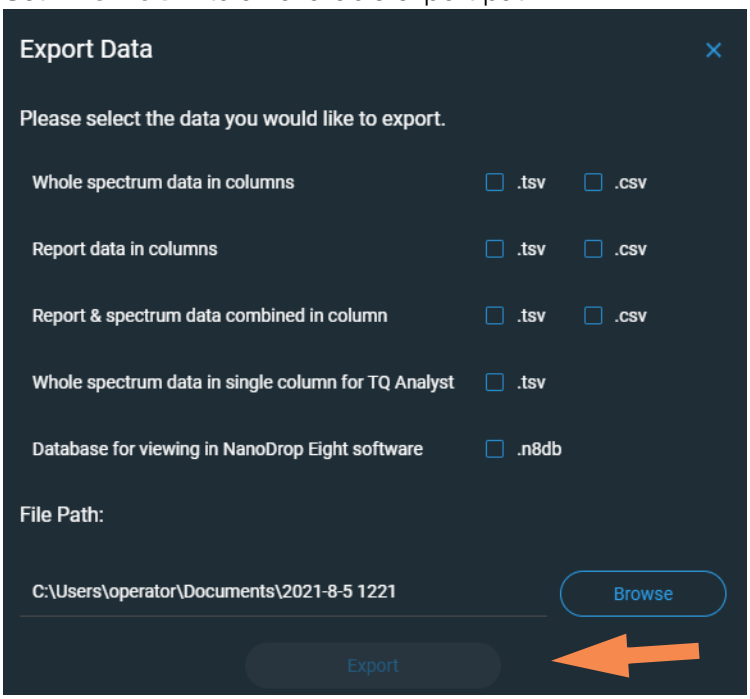
Export data at end of experiment

- when finished measuring samples, click  End Experiment
- from End Experiment box, enter Tags and edit experiment name if desired, and clock Save
- Once the experiment is saved, you can click **Export**



- select one or more formats to export to (see above for details)

– Set **File Path:** to an available export path



– click **Export**

Export data from History

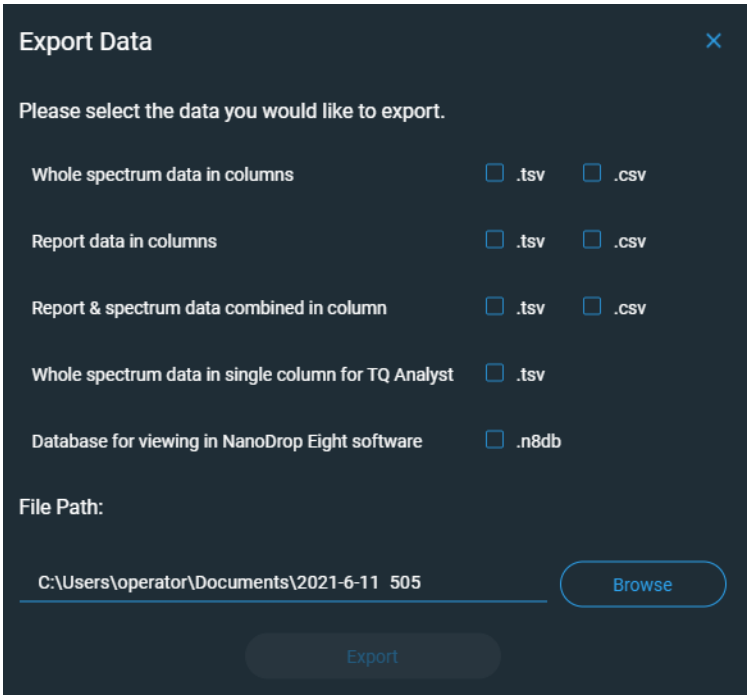
– From Home screen, open History

– Click the checkbox for the experiment(s) you want to export. You can use the Search feature to find experiment

– Click **Export**

– Select one or more formats to export to (see above for details)

– Set **File Path:** to an available export path and click **Export**



– after “Export Success” message, click **OK**

Delete Selected Measurements

You can delete selected sample measurements from any experiment, or all the measurements in the database.

Note Deleted data cannot be recovered.

Delete data from any measurement screen

– click a sample measurement row

– click 

Delete data from History

– from Home screen, open History

– select **row** in History. You can filter experiments by date or type, or use Search feature to find desired experiment

– click to select one or more experiments to export

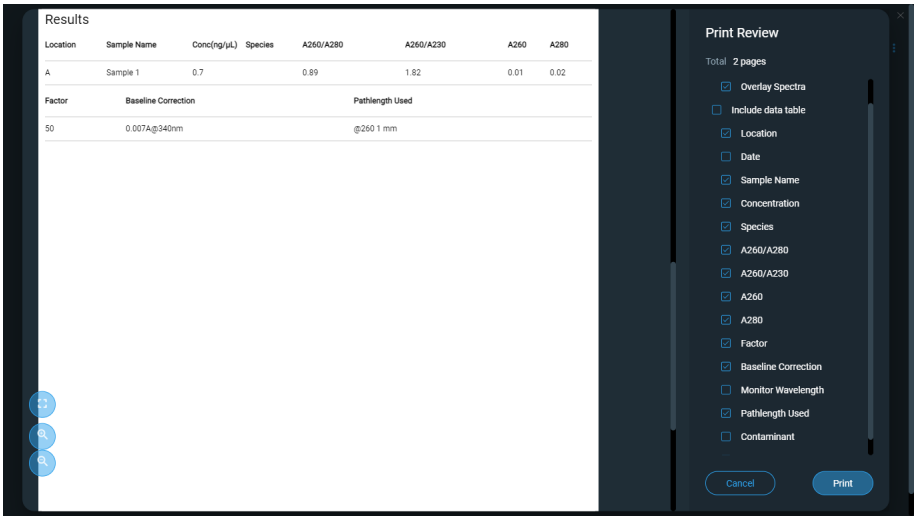
– click **Delete**

Print Selected Measurements

Connect a compatible printer to the PC to quickly print measurement results, including spectral data, standard curves, data tables, sample details and diagnostic results.

Print data from any measurement screen

- after you have measured a sample, display the measurement results to be printed such as the spectral data, standard curve, data table or sample details (see NanoDrop Eight Measurement Screens)
- from the options menu, select **Print**
- In Print Review, select the data you want to include in the data tables.



- choose **Print** to confirm
- in the Print Preview window, make sure the correct printer is selected and set other print options as desired such as paper size and orientation (“Auto” setting is recommended), margin and alignment to adjust the image in the preview window

> **Note** The software saves the print settings each time you print.

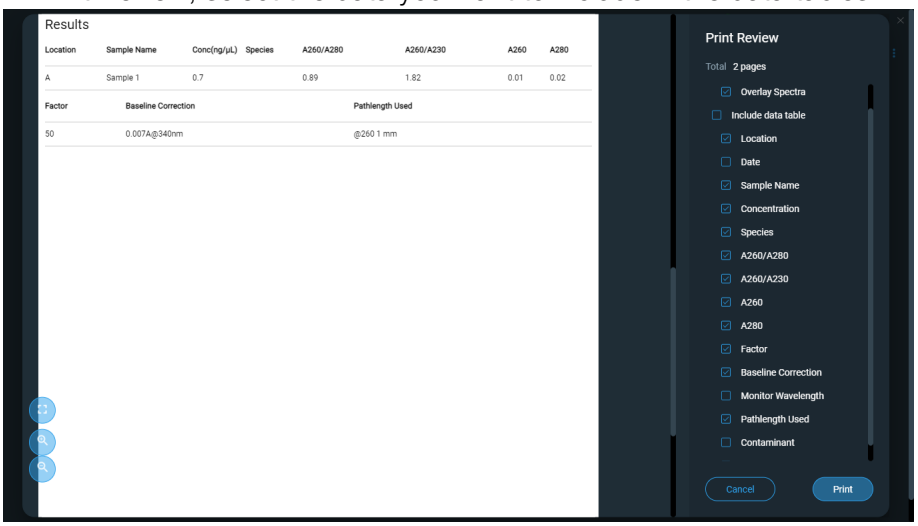
- choose **Print**

The selected measurement screen is printed for each selected measurement.

Print data from History

- from Home screen, open History
- click a **row** in History or use Search feature to find desired experiment
- click the **experiment name** to open the experiment
- click the menu in the upper right corner of the screen and choose **Print**

– In Print Review, select the data you want to include in the data tables.



– choose **Print** to confirm

– in the Print Preview window, make sure the correct printer is selected and set other print options as desired such as paper size and orientation (“Auto” setting is recommended), margin and alignment to adjust the image in the preview window

> **Note** The software saves the print settings each time you print.

– choose **Print**

The selected measurement screen is printed for each selected measurement.

Acclaro Sample Intelligence

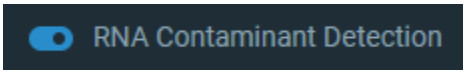
The Thermo Scientific™ Acclaro™ Sample Intelligence technology built into the NanoDrop Eight instrument provides these exclusive features to help you assess sample integrity:

- contaminant analysis to help qualify a sample before use in downstream applications
- on-demand technical support for measurements that are atypical or very low concentration

Use these embedded resources to quickly troubleshoot possible problem measurements and make informed decisions on whether to use, re-purify or take other actions with an atypical sample result. The Sample Intelligence feature also serves as a resource for further study and a learning tool for new or novice users.

Activate Detection

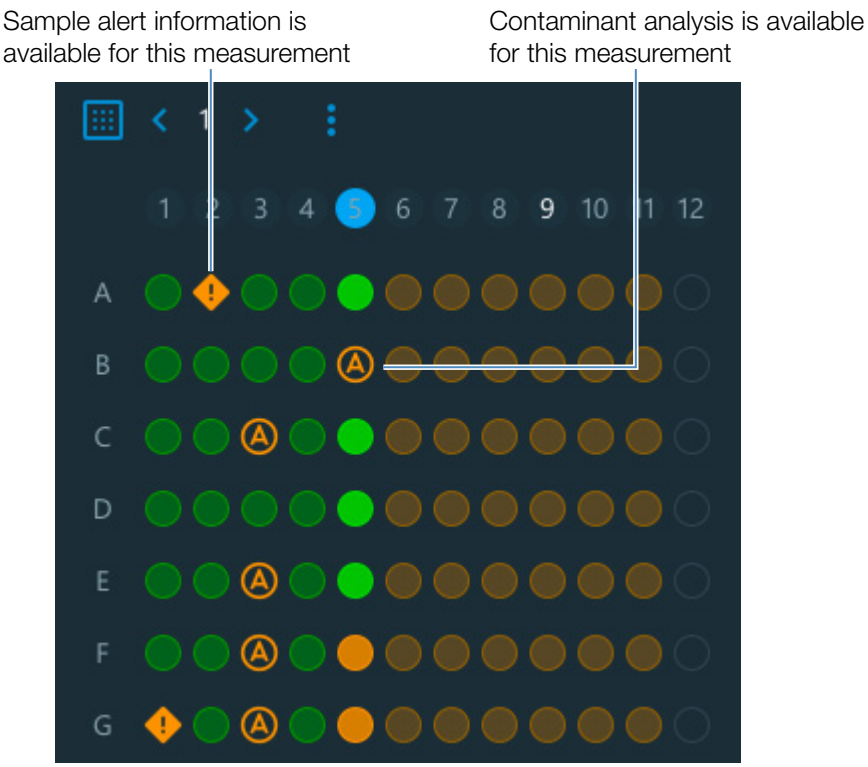
From the measurement screen, click the activate contaminant detection option.



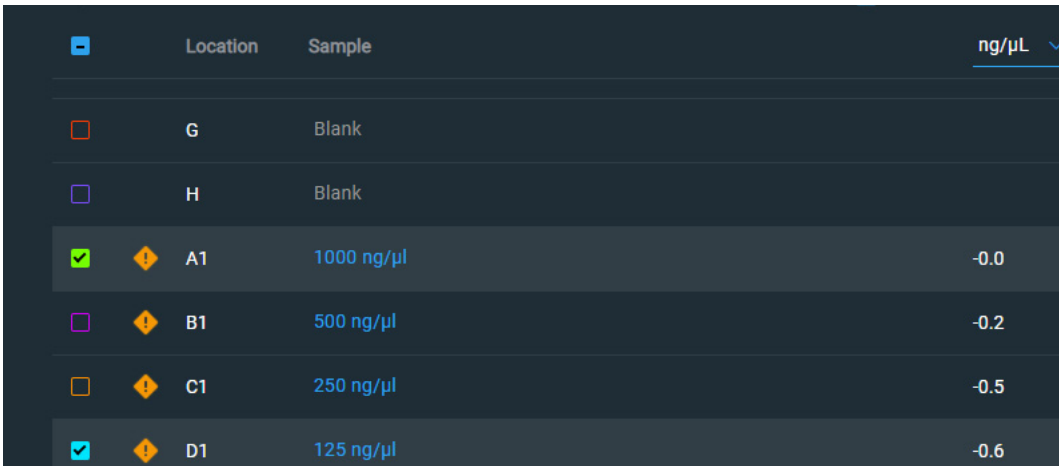
When enabled, RNA Contaminant Detection/DNA Contaminant detection will apply mathematical models to predict the amount of RNA contaminant in dsDNA or dsDNA in RNA. These models are specific to the source of the nucleic acid. The mouse icon is representative of all mammalian sources of nucleic acid. If you are measuring nucleic acid from a source for which we do not have mathematical model, leave the selections unchecked.

View Acclaro Sample Intelligence Information

Measurements that include a contaminant analysis or technical information are flagged automatically (see examples below). Select the icon to review the associated data or information.



The icons appear in the Sample Plate Map, the data table, and in History (see below).



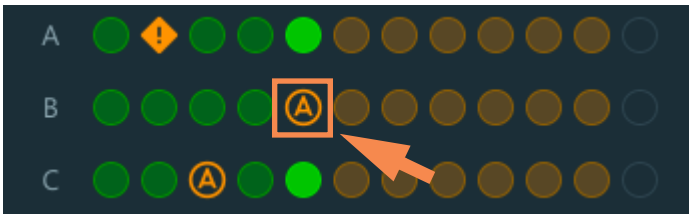
The icons are active in all three places; the information remains with the data indefinitely, even after it has been exported.

Contaminant Analysis

For the dsDNA, RNA and Protein A280 applications, the NanoDrop Eight software automatically initiates a spectral analysis for several known contaminants during the measurement. Examples of known contaminants include:

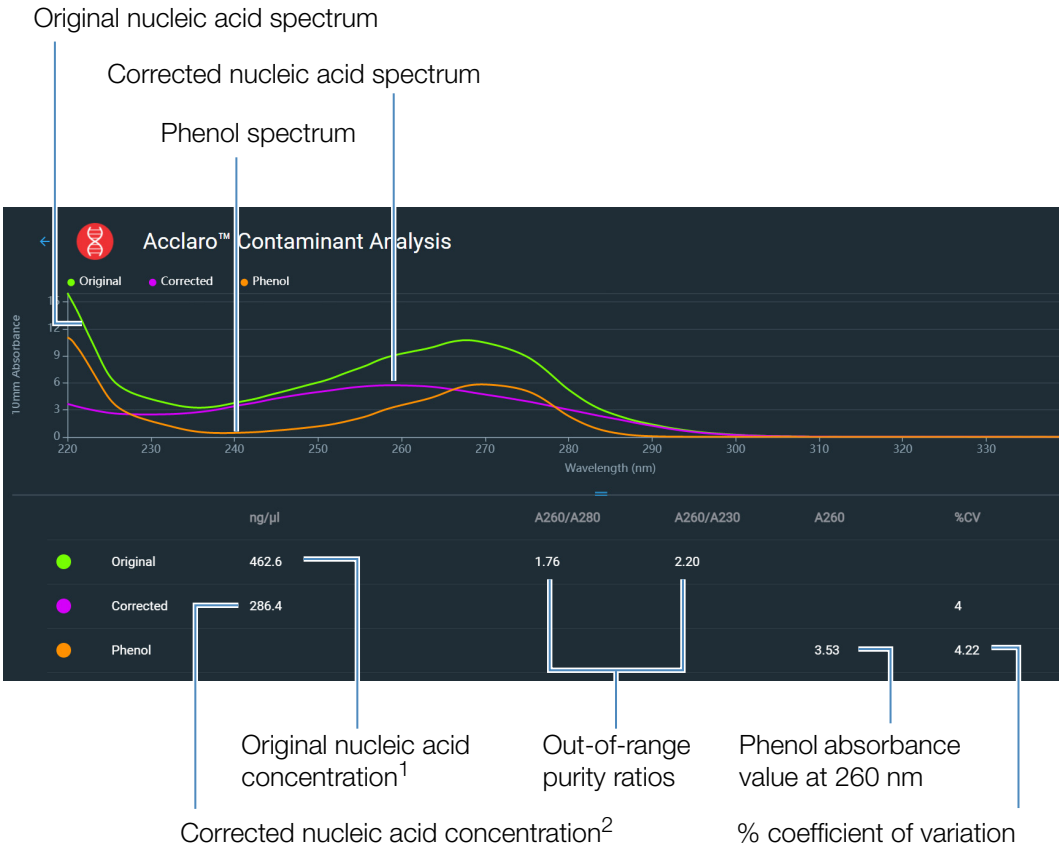
- for dsDNA and RNA measurements:
  - in the analysis region: protein and phenol
  - detects presence of guanidine HCl and guanidinium isothiocyanate
  - detects species-specific dsDNA contamination in the RNA application and detects species-specific RNA contamination is in the dsDNA application
- for protein measurements:
  - in the analysis region: nucleic acids and phenol

If contaminants are identified in a sample, the “Contaminant Analysis” icon appears to the left of the measurement results.



Click the icon to view the contaminant analysis and associated information.

The following is an example of results from a nucleic acid contaminant analysis that contains enough protein contaminant to affect the measurement results.



[1]Based on total sample absorbance (sample plus contaminant)

[2]Based on corrected sample absorbance (sample minus contaminant)

Since phenols absorb light near the analysis wavelengths for nucleic acid (230 nm, 260 nm, and 280 nm), the presence of phenol in the nucleic acid sample shown above has pushed the A260/A280 and A260/A230 ratios out of range and caused the reported nucleic acid concentration to be higher than the real value. The software identifies the impurity (phenol), and reports the following:

- baseline-corrected absorbance due to phenol (2.53) at the analysis wavelength (260 nm)
- % coefficient of variation for the measurement result (uncertainty x 100/measurement result = 4.22%; a high %CV indicates the measurement result is close to the instrument detection limit or there is an interfering component)
- original nucleic acid concentration (462.6 ng/µL), which is based on the total baseline-corrected absorbance (sample plus contaminant) at the analysis wavelength
- corrected nucleic acid concentration (286.4 ng/µL), which is based on the corrected absorbance (sample minus contaminant) at the analysis wavelength

Theory behind contaminant analysis

UV and UV-visible absorbance measurements are used to quantify nucleic acid and protein samples at 260 nm and 280 nm, respectively. The analysis is based on the fact that the total absorbance of a mixture solution at a given wavelength is the sum of the absorbance values of each component in the mixture.

An ongoing challenge of this method is that a number of materials used in the extraction process can absorb in various regions across the spectrum. When these contaminants are present in a sample, they can interfere with the analysis by artificially inflating the absorbance at the wavelength of interest, which causes the analyte concentration to be overestimated.

Traditionally, purity ratios are used to detect the presence of contaminants that could affect downstream applications. However, purity ratios do not always provide a complete picture of possible contamination. When a purity ratio falls outside the expected range, the spectral profile is often examined qualitatively.

Our Acclaro technology applies a quantitative approach to contaminant analysis. Through sophisticated mathematical algorithms, Acclaro analyzes the spectral data to identify probable contaminants in a sample and removes any contribution due to the contaminant from the sample result. This results in a more accurate concentration value of the analyte of interest and a more quantitative analysis of the level of contamination.

Since the spectrum of a pure compound is unique to that compound, a mixture spectrum of mostly known materials that have few interactions can be mathematically broken down into its component spectra and the components identified. The contaminant analysis algorithm uses a narrow spectral region (220-285 nm) around the analysis wavelength (260 nm for nucleic acids, 280 nm for proteins) to determine any absorbance contribution from possible known contaminants (protein or nucleic acid, and phenol) that absorb in that region. The entire spectrum is analyzed to determine the presence of other possible contaminants such as guanidine HCl and/or guanidinium isothiocyanate, which are common reagents used for nucleic acid purification.

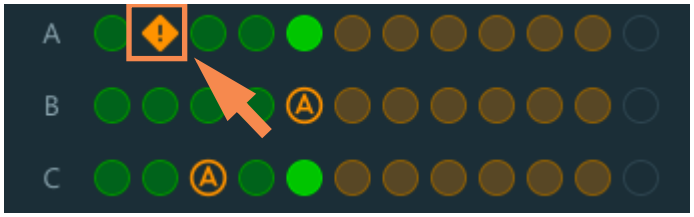
Note Achieving consistent, high quality contaminant analysis results is dependent on the quality of the measured sample spectra, which is dependent on the maintenance status of the instrument. For more information, see Maintenance Schedule.

On-Demand Technical Support

For the dsDNA and Protein A280 applications, the NanoDrop Eight software monitors all sample measurements for the presence of contaminants or other anomalies that may affect the measurement. Examples of monitored characteristics include:

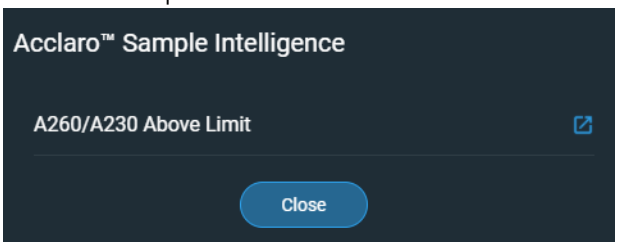
- absorbance ratios, which indicate the presence of compounds that may interfere with sample measurements (also referred to as “purity ratios”). For more information, watch the multimedia training What is a Purity Ratio?.

If sample alert information is available,  appears to the left of the measurement results.



Click the icon to view the information.

Here are results from a nucleic acid analysis for which measured purity ratios are above the expected value



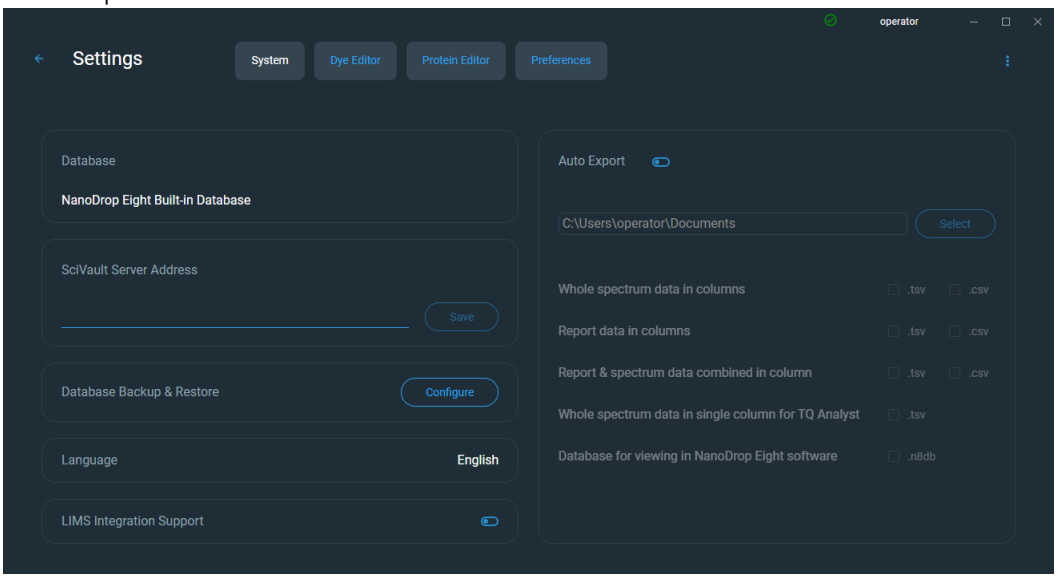
Click the  **More Information** icon for the next level of information

Instrument Settings

From the Home screen, select Settings. Available options include System Settings, Dye Editor, Protein Editor, and Preferences.

System Settings

These options are available:



Database — The NanoDrop Eight uses a built-in database

Security — Used when the optional Thermo Scientific™ SciVault™ software is installed. The server location is automatically populated for single computer installations, but must be Copy and Pasted for different computer installations.

Database Backup & Restore — Set database backup and restore options such as file path and data and backup schedule.

Language — Select language for displaying NanoDrop Eight software.

Notice: Changing the language requires a software restart.

Auto-Export — Select a file path for exported files and enable/disable Auto-export at the end of experiments

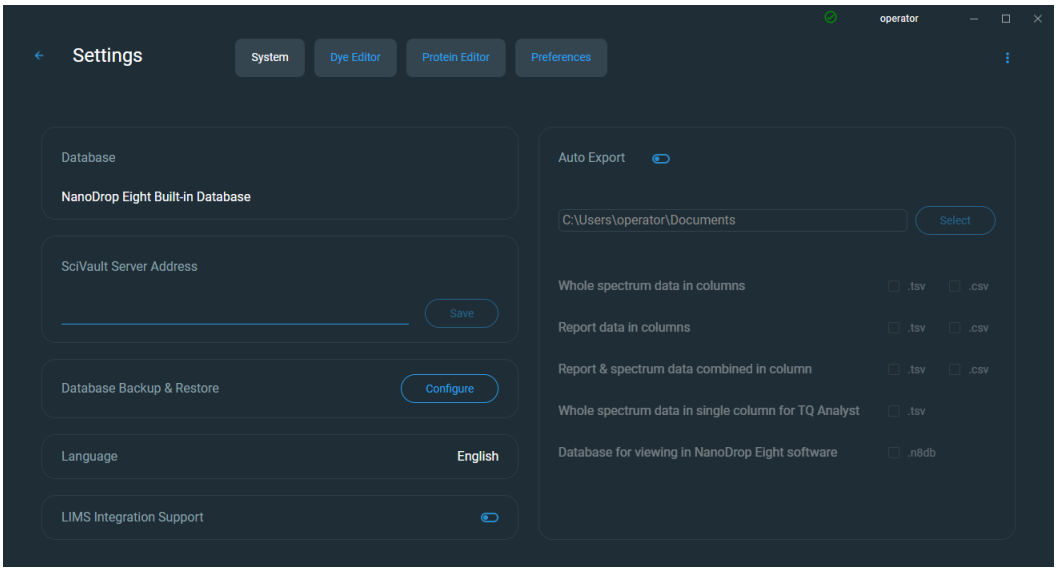
LIMS Integration Support — Enable when importing data into LIMS software. Incorporates a checksum into the exported file.

Database Backup and Restore

You can backup and restore the measurement database to a specified location of your choosing.

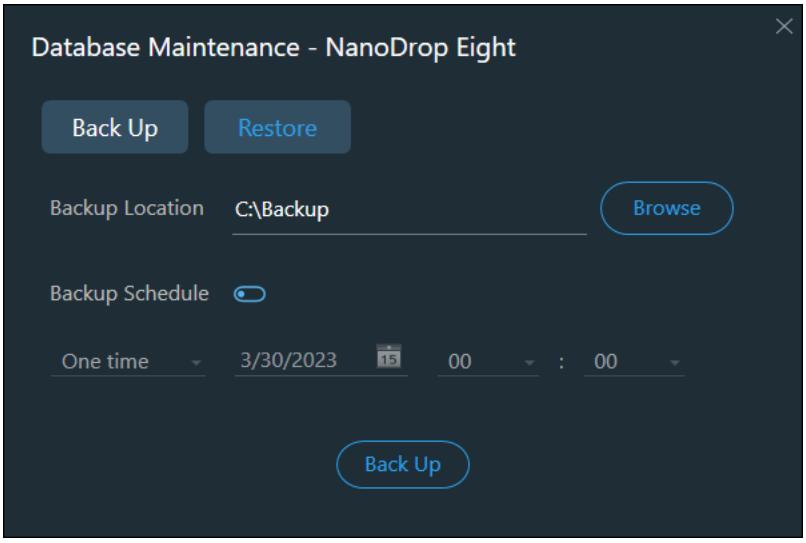
Configure database backup

From the **Settings** screen, in the **Database Backup & Restore** region, select **Configure**.

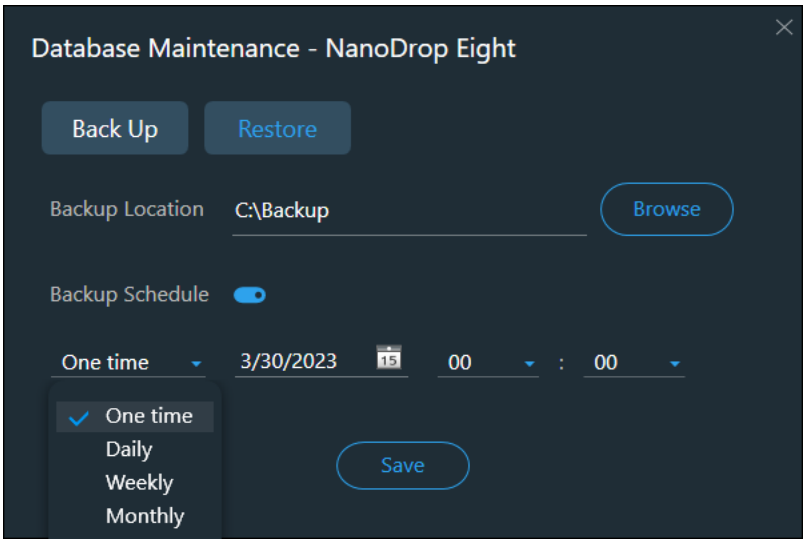


The current backup location is displayed.

Click **Browse** to assign the backup location.



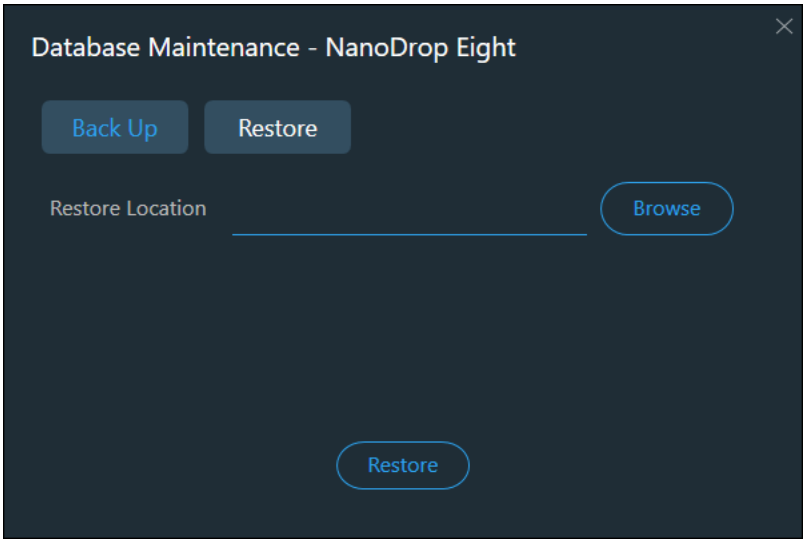
You can configure a database backup as a one-time event, or as a periodic scheduled backup. Specify the date and time of a one time backup, or toggle **Backup Schedule** to then select the period of the backup from the drop-down menu.



Select **Back Up** to save the database to the backup location.

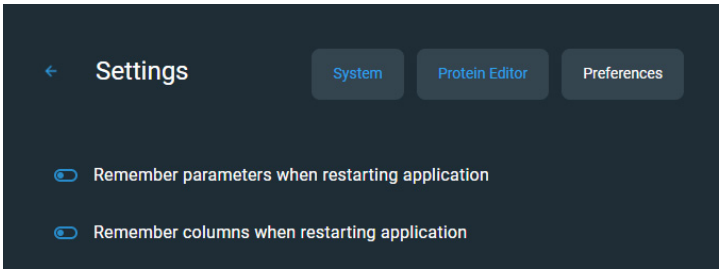
Restore database from backup

To restore data from a previous backup event, from Database Maintenance, select **Restore** and browse for the database backup you wish to use.



Once you have selected your backup, click the **Restore** button.

Preferences



Set your preferences for remembering certain settings when restarting the application.

Dye Editor

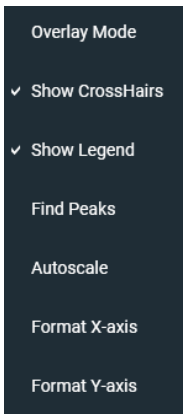
See “Dye/chromophore editor” on page 32 for information on using the dye editor.

Protein Editor

See “Protein editor” on page 63 for information on using the protein editor.

Measurement Screen Display Options

When performing measurements using the PC Control software, right-click the graph on the measurement screen to bring up the following display options:



Display multiple samples in overlay.

Hover spectrum to see plot data

Display graph legend

Calculate peaks for specified range

Scale axes to fit spectra measurement

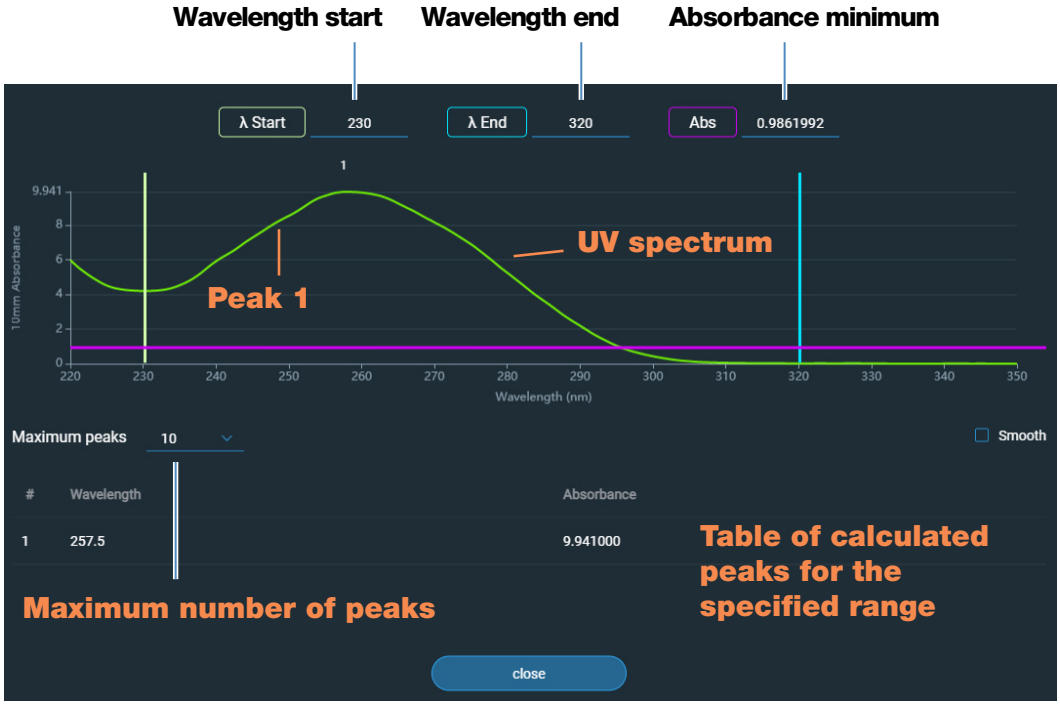
Select to manually enter x-axis range

Select to manually enter y-axis range

Display options- Right click graph to view

Find Peaks

Select **Find Peaks** to view calculated peaks for specified range. You can enter the range by dragging the color-coded limit lines, or enter values into the fields at the top of the spectrum. Found peaks for the defined range are listed in the table below the spectrum.



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9

Maintenance

Maintenance Schedule

Daily Maintenance

- Clean pedestals with deionized water

Periodic Maintenance

- Clean and recondition pedestals

Every 6 Months

- Clean and recondition pedestals
- Run Intensity Check
- Run Performance Verification

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

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

Maintaining the Pedestals

The pedestals require periodic maintenance to maintain measurement integrity. Time lines and procedures for cleaning and reconditioning the pedestals are provided below.

Cleaning the Pedestals

To avoid carryover and cross contamination, clean the pedestals before the first blank or sample measurement and at the end of each measurement. Additional cleaning (see below) or reconditioning may be required for periodic maintenance.

Note

- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids may flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestals as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

Note Solutions containing detergent or isopropyl alcohol may uncondition the pedestals. If these are required for sample analyses, follow immediately with 3–5 µL DI H_2O.

Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- for thorough cleaning: PR-1 kit

To clean the pedestals between measurements

Lift the instrument arm and clean the upper and lower pedestals with a new laboratory wipe.

To clean the pedestals between users

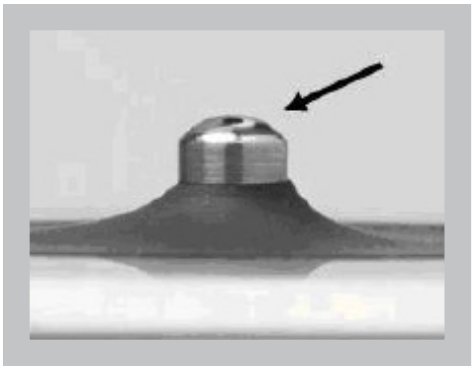
1. Lift the arm and clean all pedestals with a new laboratory wipe.
2. Pipette 3 µL DI H_2O onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean all pedestals with a new wipe.

Tip: When thorough cleaning is required (for example, to remove dried sample left on the pedestals), clean and recondition the pedestals using PR-1 compound. If you do not have PR-1, you can also substitute 0.5M HCl for the DI H_2O in the procedure above and follow with 3 uL DI H_2O.

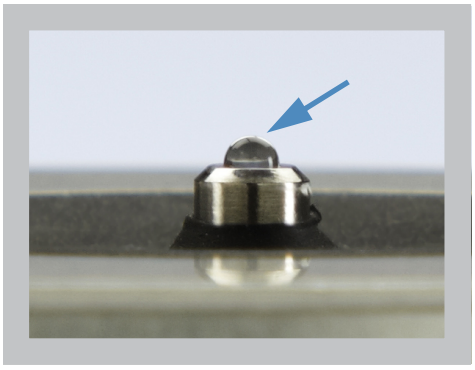
Reconditioning the Pedestals

The pedestal surfaces may lose their “conditioned” properties over time, especially after measurements with isopropyl alcohol or solutions that contain surfactants or detergents such as the Bradford reagent. An unconditioned pedestal causes droplets on the lower pedestal to “flatten out,” preventing proper formation of the liquid column when the arm is lowered. The resulting spectrum may look “rough” or “jagged.”

If samples flatten out on the pedestal (rather than “beading up” or forming a rounded droplet) or the liquid column breaks during a measurement, recondition the pedestals.



Unconditioned pedestal
(droplet flattens out)

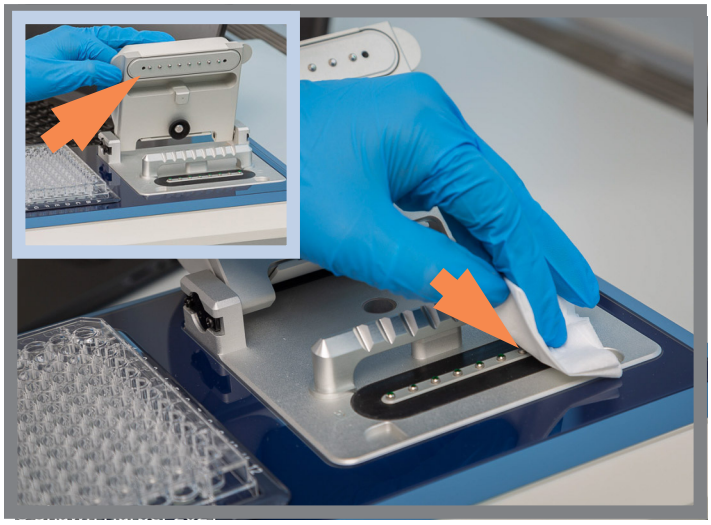


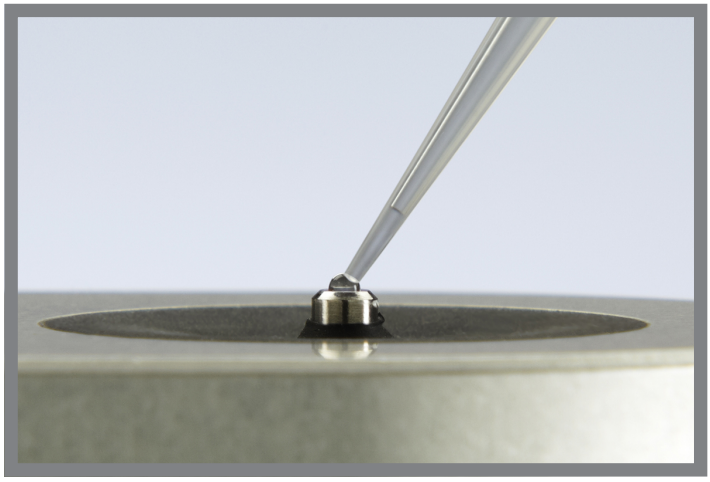
Properly conditioned pedestal
(droplet beads up)

Supplies needed

- lint-free laboratory wipes
- PR-1 pedestal reconditioning kit (available from us or a local distributor)
- calibrated precision pipettor (0-2 µL)
- canned air

To recondition the pedestals





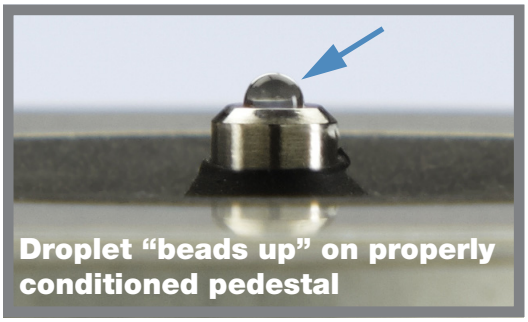
1. Open the container of PR-1 compound and use the provided applicator to remove a pin-head sized amount of the compound.
2. Apply a thin, even layer of reconditioning compound to the surface of the upper and lower pedestal.

   Wait 30 seconds for the PR-1 compound to dry.
3. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal.

   Notice: Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.

   Tip: Black residue on the wipe is normal.
4. Repeat step 3 with a new folded wipe until all residue is removed and the pedestals buff clean.
5. Use canned air to remove any paper residue from the pedestals.
6. Pipette 1 µL DI H_2O onto the lower pedestals.

   The DI H_2O should “bead up” or form a rounded droplet.


Droplet “beads up” on properly conditioned pedestal

Tip The PR-1 pedestal reconditioning compound is the easiest way to recondition the pedestals. If you don't have a PR-1 kit, follow these steps:

1. Lift the instrument arm and pipette 3 µL 0.5M HCl onto the lower pedestals.
2. Lower the arm and wait 2–3 minutes.
3. Lift the arm and clean all pedestals with a new laboratory wipe.
4. Pipette 3 µL DI H_2O onto the lower pedestals.
5. Lower the arm and wait 2–3 minutes.
6. Lift the arm and clean all pedestals with a new wipe.

   NOTICE: Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.
7. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal at least 50 times.
8. Use canned air to remove any paper residue from the pedestals.

Decontaminating the Instrument

Decontaminate the instrument after measurements with samples that contain hazardous materials and before returning the instrument to us for maintenance or repair.

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

Note

- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids may flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestals as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.
-

Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- 0.5% sodium hypochlorite solution (1:10 dilution of commercial bleach, freshly prepared)
- pipettor

To decontaminate the pedestals

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Pipette 2–3 µL diluted bleach solution (see Supplies needed) onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean all pedestals with a new wipe.
5. Pipette 3–5 µL DI H_2O onto the lower pedestal.
6. Lower the arm and wait 2–3 minutes.
7. Lift the arm and clean all pedestals with a new wipe.

To decontaminate the instrument surfaces

1. Dampen a clean, soft cloth or laboratory wipe with the diluted bleach solution (see Supplies needed) and use it to gently wipe the outside surfaces of the instrument.
2. Use a clean cloth or wipe dampened with DI H_2O to remove the bleach solution.

Instrument Diagnostics

Every 6 months, run the following performance and quality checks to verify instrument operation.

Intensity Check 210

Performance Verification 212

Diagnostics can be performed using the NanoDrop Eight software. **Intensity Check** and **Performance Verification**, are all accessible from the software Home screen:

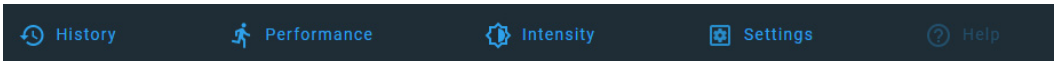


Figure 1. Control options

History:	View data stored locally. Filter by date or application.
Performance:	Performance verification process using PV-1 solution
Intensity:	Run an intensity check for the pedestal
Settings:	Set security server location and path if desired
Help:	View help

Intensity Check

Run Intensity Check every 6 months to verify operation of the instrument's internal components. The test measures the intensity of light from the xenon source through the instrument to verify that throughput, wavelength accuracy, and bias are within specifications. The test is automatically performed using the pedestal paths.

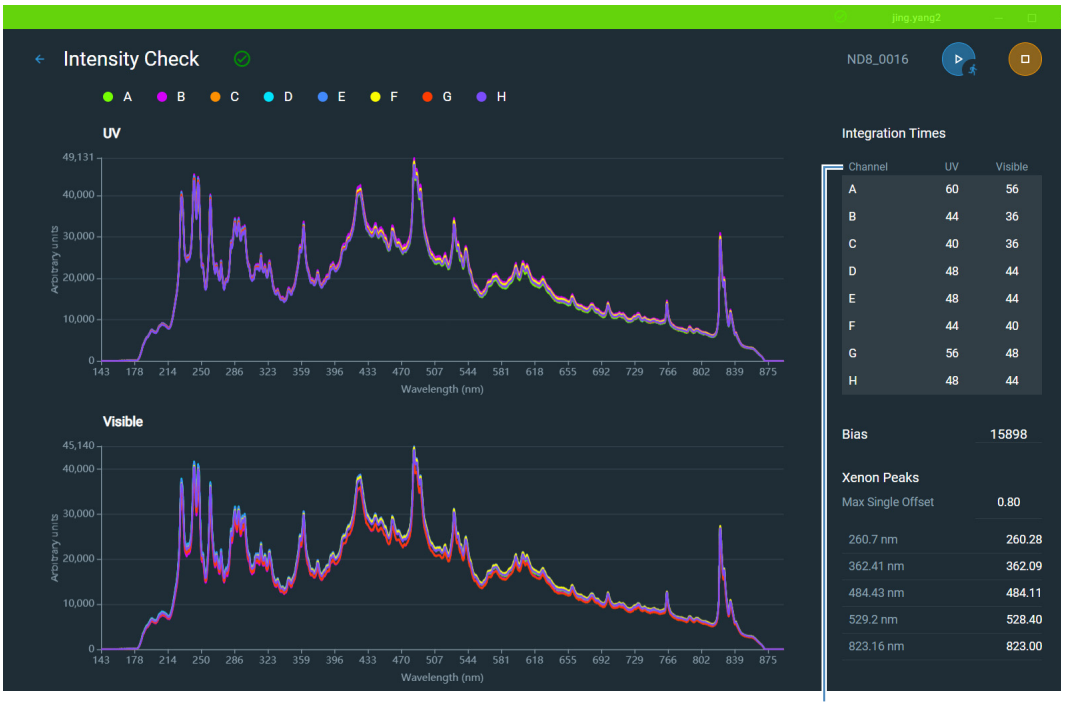
Supplies needed

- lint-free laboratory wipes

To run intensity check

1. From the Home screen, select **Intensity**.
2. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
3. Click **Ready** and lower the arm.
4. If **Auto-Measure** feature is OFF, click **Measure** to begin the measurement. If **Auto-Measure** feature is ON, the measurement will begin automatically after the arm is lowered.

Here is an example of a typical intensity check result screen.



Channel table

5. To rerun the intensity check, select **Run**.
6. When finished, select **End Experiment**.

   After the test is completed, the results are available from the History. See Manage identifiers on the instrument for details.

To interpret intensity check results

The results will display the lamp intensity using the UV and Visible integration times in two separate graphs (see image above).

Users can see the intensity graphs of a single channel by clicking a single row from the Channel table or Shift + Click to highlight multiple rows (see image to the right).

A green check mark and banner will be displayed at the top if the UV intensity, Visible intensity, bias, and maximum single offset are all within specification. If one or more is not within specification, a message will be displayed at the top indicating what parameter(s) failed. If any parameter has failed, clean the pedestals with deionized water and then repeat the Intensity Check.

If a yellow triangle appears next to the Bias indicator, make sure the room is within the temperature specifications for the instrument.

If the Intensity Check fails again, contact us.

Performance Verification

Run Performance Verification every 6 months to confirm pathlength accuracy is within specifications.

A CHEM-PV-8 kit containing two vials of PV-1 {aqueous nicotinic acid ($C_6H_5NO_2$), potassium nitrate (KNO_3)} is required to verify the performance of the Thermo Scientific™ NanoDrop™ Eight microvolume UV-Vis spectrophotometer.

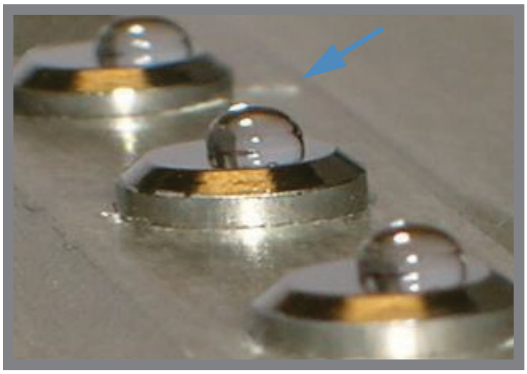
Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- Calibrated Precision 8-channel Pipettor (0.5 - 10 µL)
- CHEM-PV-8 kit containing two ampoules of PV-1, two 8-well PCR strip tubes, and transfer pipettes
- laboratory gloves

Note The PV-1 solution comes in a single-use ampoule. Before you open the ampoule, shake it vigorously and then allow the liquid to collect in the bottom portion of the ampoule. After the ampoule is opened, its contents must be used within one hour. Pipette directly from the ampoule; do not transfer the solution.

Before you begin

First make sure the pedestals are properly conditioned. To test pedestal conditioning, clean the upper and lower pedestal surfaces with a dry, lint free laboratory wipe, then pipette 1.5 µL DI H_2O onto the eight lower pedestal surfaces. The droplets should “bead up” as shown below. If it does not, recondition the pedestals. Remove the water sample from the upper and lower pedestal surfaces with a dry laboratory wipe.



Droplets “bead up” on properly conditioned pedestals

Sample Loading Hints

- Ensure the NanoDrop Eight instrument is not situated near an air vent or an exhaust fan from a nearby instrument.
- Use a small-volume (0.5 - 10 µL) 8-channel pipettor to load the di$H_2$0 and the PV-1 aliquots.

Discharge and Touch Sample Delivery Method Practice

It is very important to deliver the PV-1 aliquots to all eight pedestals in a single motion using a multichannel pipettor. It is highly recommended that the steps below be practiced using water before starting the calibration check procedure.

1. When the pipette tips are close to the measurement pedestals, discharge the fluid and allow the drops to hang on the end of the tips.
2. Gently touch the droplets to the pedestals and allow them to be pulled off the tips and onto the pedestals by surface tension.

   Tip: It is important to use tips with a rigid structure to ensure the tips do not splay or skew when delivering the samples.

   Tip: It is suggested that this technique be practiced until consistent delivery to all eight positions is routine and quick.

To run performance verification

1. Click on **Performance** at the bottom of the screen.
2. Enter the PV-1 lot number (found on the PV-1 ampoule label) into the corresponding **PV-1 Lot Number** entry box.
3. Enter Target Absorbance Value #1 (found on PV-1 ampoule label) into corresponding **Target #1 Abs** entry box.
4. Repeat step 2 for the **Target #2 Abs** entry box, using Target Absorbance Value #2 found on PV-1 ampoule label.

   Note Target Abs. values are lot specific and must be entered into the correct, corresponding entry box.

5. Once the target values have been entered, click **Continue**.
6. Add 55 µL of di$H_2$0 to each well of one of the 8-well strips provided. Do **NOT** aliquot the PV-1 at this time.
7. Use an 8-channel pipettor to pipette a 1.5 ìL aliquot of diH2O onto each of the lower pedestals, lower the arm, and click **Blank**.

   Tip: It is important to use tips with a rigid structure to ensure the tips do not splay or skew when delivering the samples.

8. Remove water from upper and lower pedestal surfaces using a clean, dry laboratory wipe.
9. Ensure PV-1 solution is thoroughly mixed by vigorously shaking both ampoules. Allow the solution to collect in the bottom portion of the ampoule, if needed gently tap the ampoule.
10. Carefully snap off top portion of each ampoule using an ampoule cracker, discard top along with ampoule cracker (use proper safety precautions for disposal).
11. Use the provided transfer pipette to dispense the contents of both PV-1 ampoules into each well of the 8-well PCR strip tube.
12. Using an 8-channel pipettor, withdraw 1.5 µL of PV-1 solution from each of the 8- wells of the PCR strip tube and pipette onto each of the 8 lower pedestals. Lower arm.

    Note If Auto-Measure feature is **OFF**, click Measure to begin the measurement. If Auto-Measure feature is **ON**, the measurement will begin automatically after the arm is lowered.

13. After the measurement is complete, remove sample from both upper and lower pedestals using a dry laboratory wipe.
14. Repeat steps 11 and 12 to measure five additional individual replicates of the PV-1 solution.
    a. Always use fresh pipette tips to remove each aliquot and use a fresh aliquot of PV-1 for each measurement.
    b. In between each measurement, remove PV-1 solution from all 16 pedestals, upper and lower, using a clean, dry laboratory wipe.
15. After each measurement is complete, the individual results will be displayed on screen and subsequently added to the existing results.
16. After six replicates have been measured, the **Status** columns for each pathlength will indicate whether the channel passed the Performance Verification.

To interpret performance verification results

1. Results will display  for Pass,  for Conditional Pass, and  for Fail.
    a. If results are not within specifications, repeat the procedure using 2 µL aliquots of PV-1.
    b. If results fail to meet specifications using 2 µL aliquots, contact support or local distributor for assistance.
2. Click **End Experiment** when done.
    a. Results can be exported and printed at this time or later from the History.

b. The Experiment name can be changed at this time and Identifiers can be added.

3. Click **End Experiment** when done.

4. To review results from a previous Performance Verification check, select **History** from the Home screen and locate the Performance Verification check results from the list of experiments.

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10

Safety and Operating Precautions

Contents

Note Be sure that all persons operating this system read the safety manual first.

Operating Precautions

CAUTION Do not remove the instrument cover. Removing the cover exposes the operator to sharp edges and delicate fiber optic cables. The instrument warranty is void if the cover has been removed.

NanoDrop Eight spectrophotometers are designed to operate indoors in an environment that meets our specifications. For details, see the site preparation guide for your instrument.

Follow these precautions to avoid damaging your NanoDrop spectrophotometer during use:

- Use a grounded power cord appropriate for your electrical service. If the supplied power cord is incompatible or if it becomes damaged, contact us.
- Do not remove the instrument cover.
- Use solvents that are compatible with the instrument (see Hazardous Materials)
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids may flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestals as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

Safety Information

Before operating a NanoDrop Eight instrument, please read the safety information and follow its recommendations for the system.

Safety and Special Notices

In many cases, safety information is displayed on the instrument itself. The symbol indicates that there is additional safety information in the documentation and failure to heed the safety precautions could result in injury.

WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

Note Follow instructions with this label to avoid damaging the system hardware or losing data.

Note Contains helpful supplementary information.

The following table lists some of the safety symbols and their indications that may appear in the user documentation.

Symbols	Indication
	This is a mandatory action symbol. It is used to indicate that an action shall be taken to avoid a hazard.
	This is a prohibition symbol. The graphic in this symbol is used to alert the user to actions that shall not be taken or shall be stopped.
	This is the general warning sign. Failure to heed the safety precautions could result in personal injury.
	Avoid shock hazard. If you see either of these symbols, there is a risk of electrical shock in the vicinity. Only qualified persons shall perform the related procedures.
	Avoid fire hazard. Do not test flammable or explosive samples. Read and follow the associated instructions carefully.
	Avoid eye injury. If you see these symbols, there is a risk of exposure to ultraviolet light, which can harm your eyes if safety glasses are not worn.
	Avoid Biohazard. This icon informs of a biological hazard in the area. Read and follow the associated instructions carefully.
	Avoid chemical burns. This symbol alerts you to possible skin irritation. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose of waste.

Symbol	Description
	Alternating current
	Earth terminal or ground
	Direct current
	Protective conductor terminal
	Frame or chassis terminal
	Fuse
	Power on
	Power off

When the System Arrives

WARNING Avoid personal injury. If this equipment is used in a manner not specified in the accompanying documentation, the protection provided by the equipment may be impaired.

CAUTION Avoid personal injury. Perform *only* those procedures described in the documentation. If there are other problems, contact us. Any other service must be performed by trained personnel.

CAUTION Avoid shock hazard. Do not remove the cover of the instrument. All service to the instrument must be performed by trained personnel.

When the instrument arrives, check the exterior of the shipping box for signs of damage. If damage is apparent, contact us or your local distributor for instructions.

- Move the shipping box to the installation location at least 24 hours before installation.

  Note

  - Inside the shipping box, the instrument is sealed in a plastic bag to keep the unit dry.
  - Allow 24 hours for the instrument to reach room temperature before opening the bag. If the bag is opened before the instrument reaches room temperature, moisture could condense on the optical components and cause permanent damage.

- Keep the instrument upright at all times.

The warranty will not cover:

- Damage due to improper moving techniques.
- Damage due to removing the sealed plastic bag before the instrument has come to room temperature.

Note It is important to have all system utilities installed before the instrument arrives. Utility installations must comply with all local building and safety codes.

Lifting or Moving the Instrument

To avoid risk of injury, use proper lifting techniques when lifting or moving the instrument or other system components.

Electrical Requirements and Safety

Power supplied to the system must be from dedicated, uninterrupted sources. Power must be free of voltage dropouts, transient spikes, frequency shifts, and other line disturbances that impair reliable performance.

If you suspect power quality problems at your site, or if your system will be installed in a heavy industrial environment, we recommend a power quality audit before installation. Contact us or your local electrical authority for more information.

CAUTION Avoid shock hazard.

- Only a qualified person using the appropriate measuring device shall check the line voltage, current and frequency.
- Only our trained and certified service representatives shall attempt to service a component that carries this symbol.
- If a protective cover on a system component appears damaged, turn off the system and secure it against any unintended operation. Always examine the protective cover for transport stresses after shipping.
- Even after this instrument has been disconnected from all voltage sources, capacitors may remain charged for up to 30 seconds and can cause an electrical shock.
- Do not allow liquid to run over or into any surface where it may gain entry into the instrument.
- Do not attempt to remove the cover of the instrument.

Grounding

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

Power Cords

Be sure to use an appropriate grounded power cord for your electrical service. If the power cord received is not appropriate for the electrical system in your location, or if the power cord becomes damaged, contact us.

Power Line Conditioning Accessories

A UPS reduces the probability of a system shutdown if power is lost elsewhere in the building. Power line conditioners (which ensure that your service is free from sags, surges or other line disturbances) also are available in the U.S.A. from us for 120 volt operation. Line conditioners for 220 volt operation can be purchased locally. Contact technical support for information about power conditioners and UPS.

Electrical Service Specifications

The following table lists the specifications for electrical service. Contact our service representative in your area if you have questions about the requirements.

Requirements	Specifications
Input current	5.0 A (max.)
Input voltage	100-240 VAC
Line frequency	50-60 Hz
Line disturbances	Sags, surges or other line disturbances must not exceed 10% of input voltage (even for a half cycle).
Noise	< 2 V (common mode) < 20 V (normal mode)

Power Consumption

Generally, 50% more power should be available than the entire system (including accessories) typically uses. Maximum power consumption and heat dissipation specifications for the spectrometer and accessories are shown below. The values are approximate.

Item	Power Consumption	Max. Heat Dissipation
instrument	60 W	205 Btu/hr

Fire Safety and Burn Hazards

Note Do not position the instrument so that it is difficult to operate the power switch or access the power supply and power cord.

To avoid a burn injury and the risk of fire or explosion:

- Use caution when testing flammable or explosive samples (see the “Hazardous Materials” section)
- Never block any of the vents on the instrument or its power supply
- Only use exact replacement power supplies from us

Optical Safety

This instrument was designed with a protective housing to prevent user exposure to ultraviolet light.

WARNING Avoid personal injury. Never look at the lamp while illuminated.

Hazardous Materials

Many standard spectroscopy methods are based on the use of solvents. Others involve corrosive samples or pressurized samples in a gaseous state.

Volatile Solvents and Flammable Samples

CAUTION Avoid personal injury. Do not leave solvents or flammable samples near the instrument. Be sure that the workspace is properly ventilated.

Compatible Solvents

Most solvents typically used in life science laboratories are compatible with the fiber optic pedestals of all NanoDrop spectrophotometers. However, the high vapor pressure properties of some solvents may not be conducive to small volume measurements when using the pedestal for measurements on any of the NanoDrop instruments. If you are measuring samples with high vapor pressures, use an instrument with provision for measuring samples in cuvettes.

The following solvents are compatible for use on the pedestals of all NanoDrop instruments.

Note Spillage of these solvents on surfaces other than the pedestals may damage the instrument.

- methanol
- ethanol
- n-propanol
- isopropanol
- butanol
- acetone
- ether
- chloroform
- carbon tetrachloride
- DMSO
- DMF
- acetonitrile
- THF
- toluene
- hexane
- benzene
- sodium hydroxide
- sodium hypochlorite (bleach)
- dilute HCl
- dilute HNO_3
- dilute acetic acid

It is recommended that all corrosive solvents be wiped from the pedestal immediately upon completion of a measurement. It is also recommended that the user end a series of measurements with a diH_2O sample to ensure that solvents are not inadvertently left on the pedestal.

The diaphragm around the pedestal of the NanoDrop Eight is permanently affixed to the instrument. Do not attempt to remove the diaphragm or break the seal. Avoid prolonged exposure of the diaphragm to HCl, alcohol, bleach, acetone or other solvents as the adhesive securing the seal may be affected. If the seal comes loose please contact us.

Note All forms of Hydrofluoric Acid (HF) are incompatible as the fluoride ion will etch the fiber optic cable.

Biohazard or Radioactive Materials and Infectious Agents

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Wear appropriate protective equipment. Individuals should be trained according to applicable regulatory and organization requirements before working with potentially infectious materials. Follow your organization's Biosafety Program protocols for working with and/or handling potentially infectious materials.

WARNING Reduce the risk associated with potentially infectious samples:

- Do not spill samples on any of the instrument components.
- If spill occurs, disinfect the external surfaces immediately following your laboratory protocols.

Instruments, accessories, components or other associated materials should not be disposed of and may not be returned to us or other accessory manufacturers if they are contaminated with biohazard or radioactive materials, infectious agents, or any other materials and/or conditions that could constitute a health or injury hazard to employees. Contact us if you have questions about decontamination requirements.