Custom Factor Pro

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

Measure Custom Factor Pro

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

Before you begin...

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

NOTICE

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

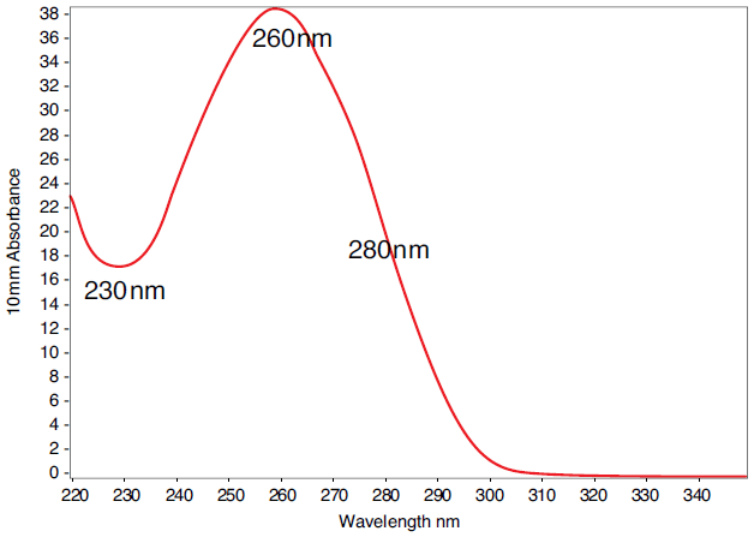
Procedure

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

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

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

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



Typical nucleic acid spectrum

Related Topics

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

Reported Results

Custom Factor Pro measurement screen (local control)

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

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

Custom Factor Pro measurement screen (PC control)

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

Settings

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

Setting	Available Options	Description
Auto Naming	On or off	When enabled, each sample is given a default base name "sample" followed by the number sample in the sequence. For example, the first sample would be named "Sample 1" followed by "Sample 2," etc. You can edit the default base name and overwrite any sample name.
Custom Factor	Enter an integer value between 15 ng-cm/µL and 150 ng-cm/µL	Constant used to calculate nucleic acid concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength: $f = 1/(\varepsilon_{260} * b))$ 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 Ultra 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.

Detection Limits

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

To calculate upper detection limits for nucleic acid samples

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

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

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

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

Related Topics

- Detection Limits for All Applications

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Oligo DNA Pro or Oligo RNA Pro

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

Measure Oligo DNA Pro or Oligo RNA Pro

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

Before you begin...

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

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

NOTICE

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

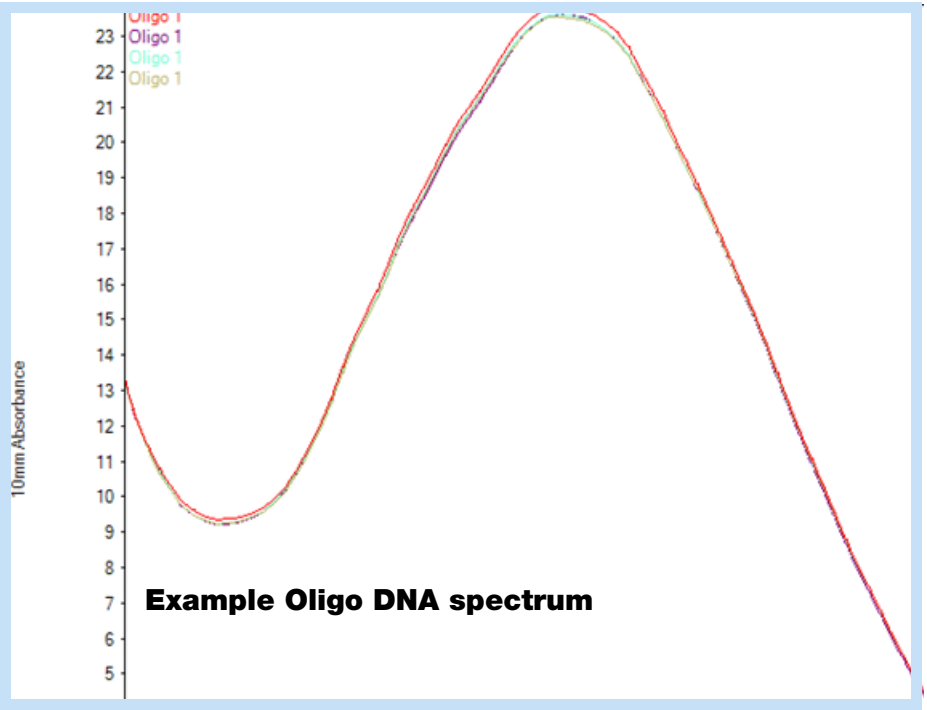
Procedure

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

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

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

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



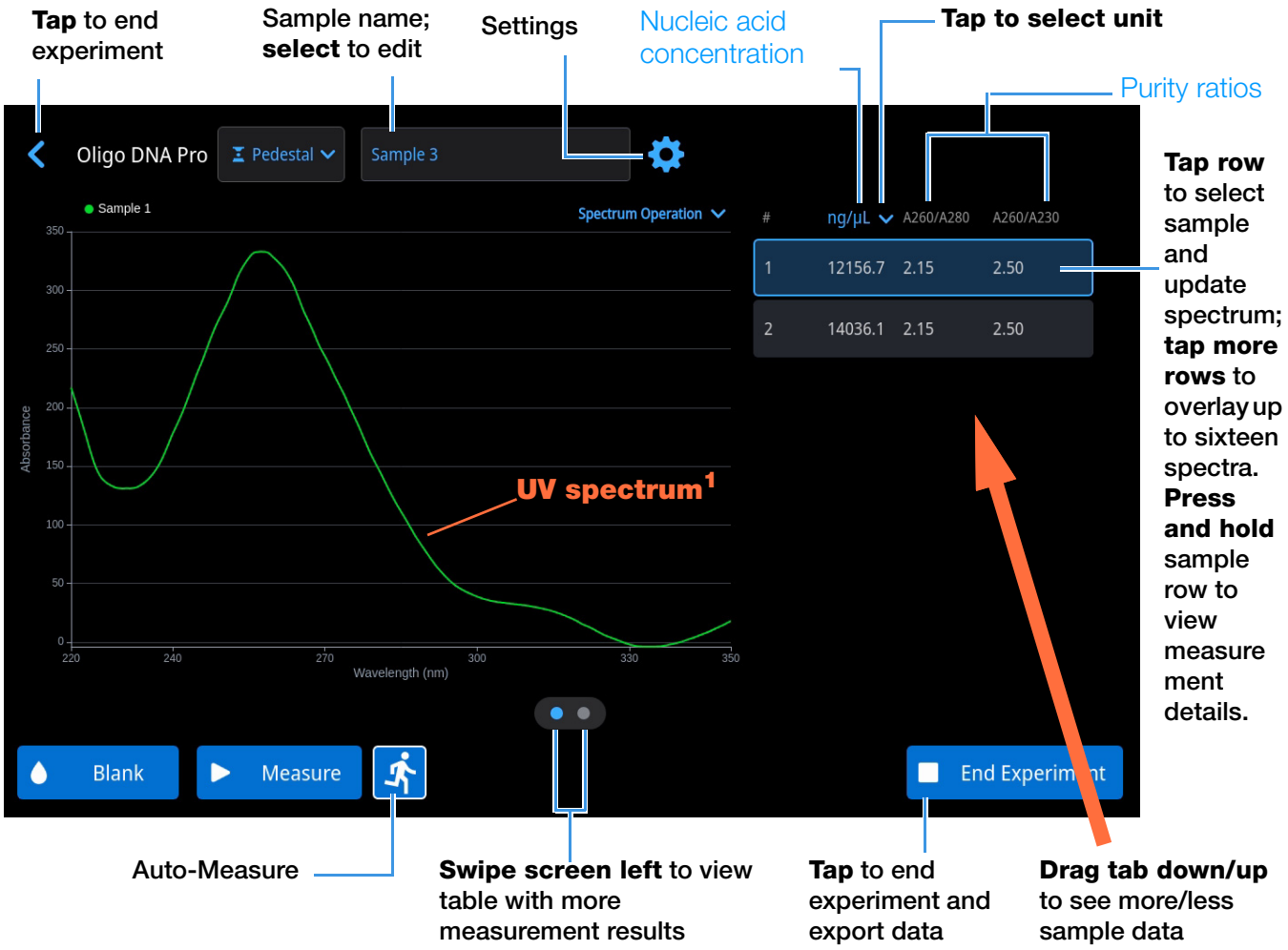
Related Topics

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

Reported Results

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

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



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

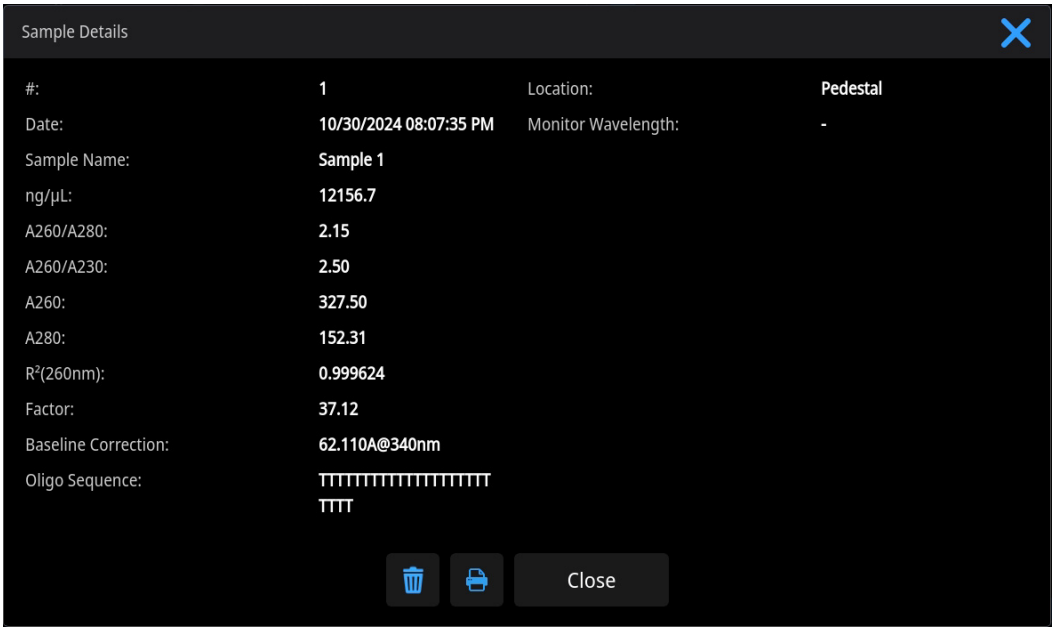
Note Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

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

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

Reported values

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



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

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

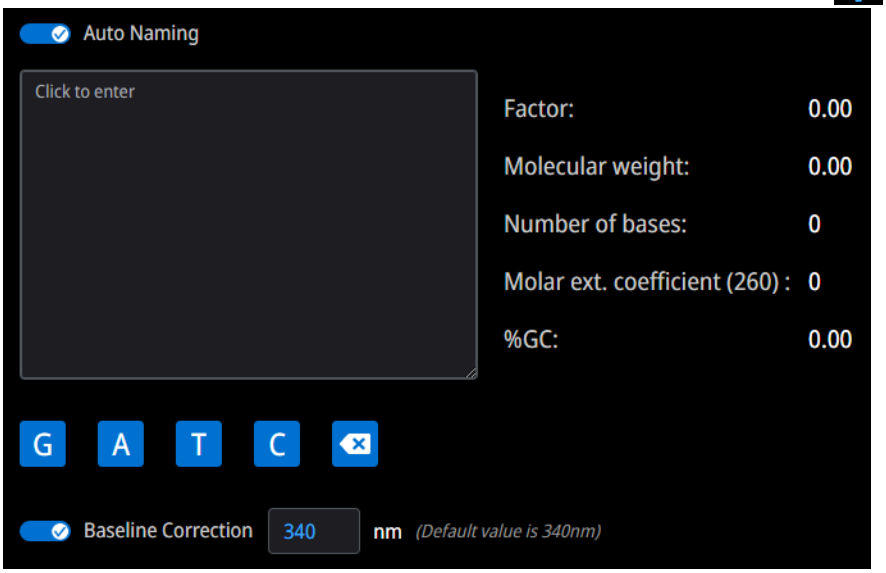
Note The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information.

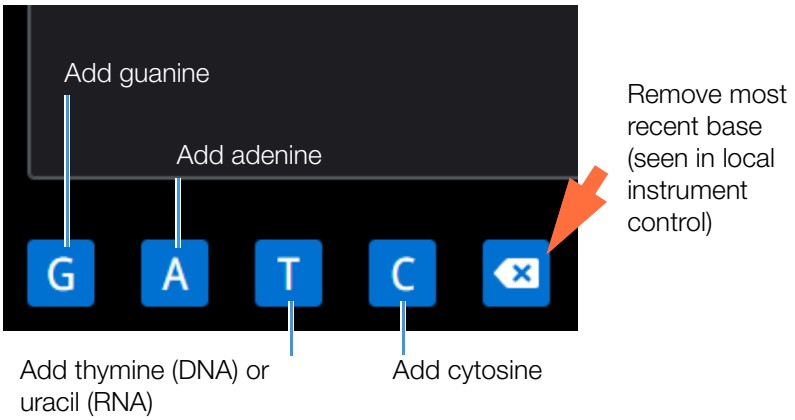
Related Topics

- Basic Instrument Operations
- Oligo Calculations

Settings

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



Setting	Available Options	Description
Auto Naming	On or off	When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name.
Oligo Base Sequence	for DNA: Use the G, A, T and C keys to specify the DNA base sequence for RNA: Use the G, A, U and C keys to specify the RNA base sequence	Specify your DNA or RNA base sequence. Tap or click the corresponding keys:  From the PC control software, you can also enter base sequence using the keyboard, or by copy and pasting a sequence from another application.
		Each time a base is added to the sequence, the software calculates the following: • **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 (0.1 cm for nucleic acids measured with the NanoDrop Ultra instrument)

Setting	Available Options	Description
		• **Molecular Weight** of oligo calculated from user-defined base sequence. • **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

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

To calculate upper detection limits for nucleic acid samples

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

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

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

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

Calculations

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

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

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

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

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

Measured Values

A260 absorbance

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

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

A230, A280 absorbance

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

Reported Values

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

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

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

Related Topics

- Calculations for Nucleic Acid Measurements

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Protein A280 Pro

More accurately measures the concentration of highly concentrated (> 62.5 A) purified protein samples that absorb at 280 nm using the Acclaro Pro enhanced automated pathlength selection algorithm.

Measure Protein A280 Pro

Reported Results

Settings

Detection Limits

Calculations

Best practices for protein measurements

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

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

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

- Choosing a blank:

  – For the Protein A280 Pro application, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.

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

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

- For micro-volume measurements:

  – Ensure pedestal surfaces are properly cleaned and conditioned. (Proteins tend to stick to pedestal surfaces.)

- Gently (but thoroughly) vortex samples before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
- Follow best practices for micro-volume measurements.
- Use a 2 µL sample volume. See Recommended Sample Volumes for more information.

Related Topics

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

Measure Protein A280 Pro

Use the Protein A280 application to quantify purified protein samples that contain amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, which exhibit absorbance at 280 nm with increased accuracy at higher concentrations (> 62.5 A). This application reports protein concentration measured at 280 nm and one absorbance ratio (A260/A280). A single-point baseline correction can also be used. This application does not require a standard curve.

Before you begin...

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

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

NOTICE

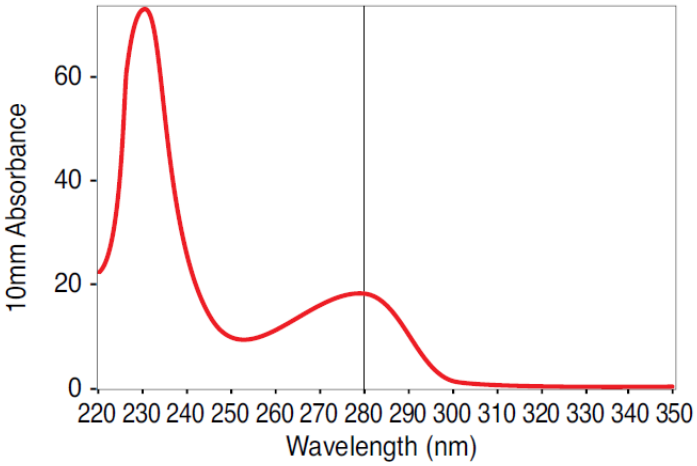
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

Procedure

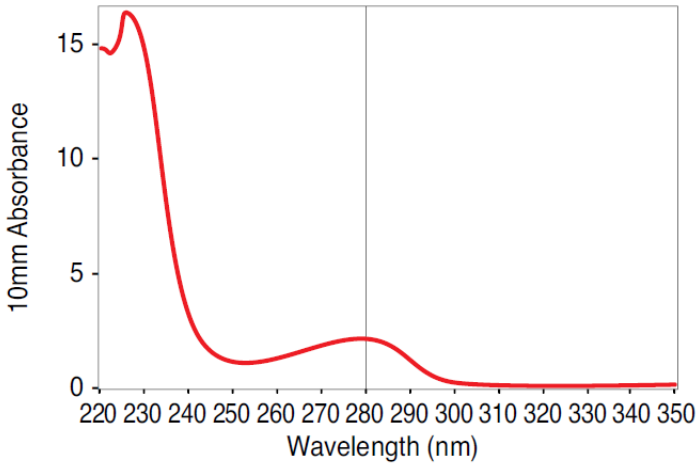
1. From the home screen, select the **Acclaro Pro** tab, then select **Protein A280 Pro**.
2. Configure any of the setup options if desired and select **Save**.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm.
4. Select **Blank** and wait for the measurement to complete.

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

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



High concentration BSA sample

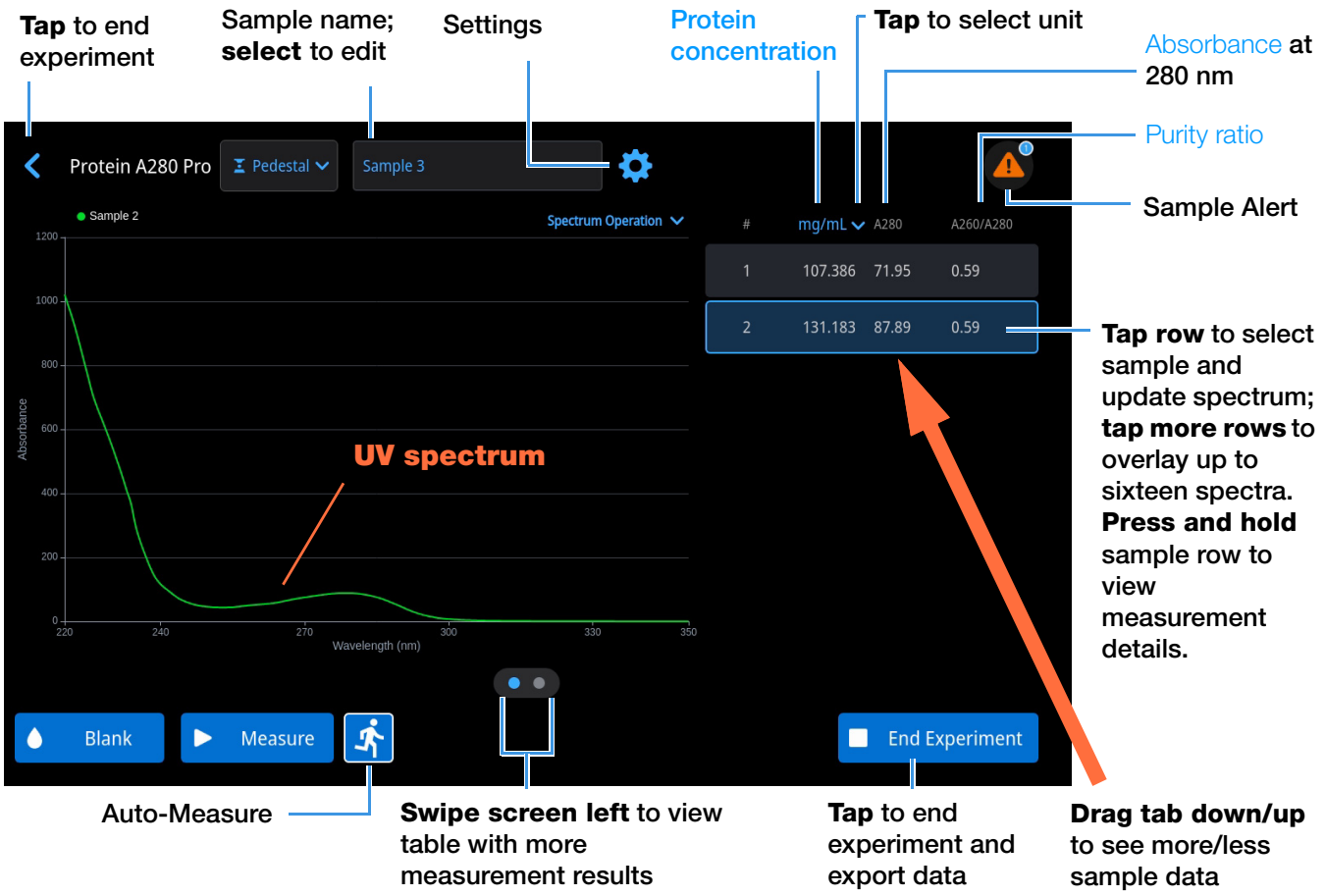


Low concentration BSA sample

Reported Results

Protein A280 Pro measurement screen (local control)

For each measured sample, the Protein A280 Pro application shows the absorbance spectrum and a summary of the results. Here is an example of the measurement screen within the NanoDrop Ultra local control software:



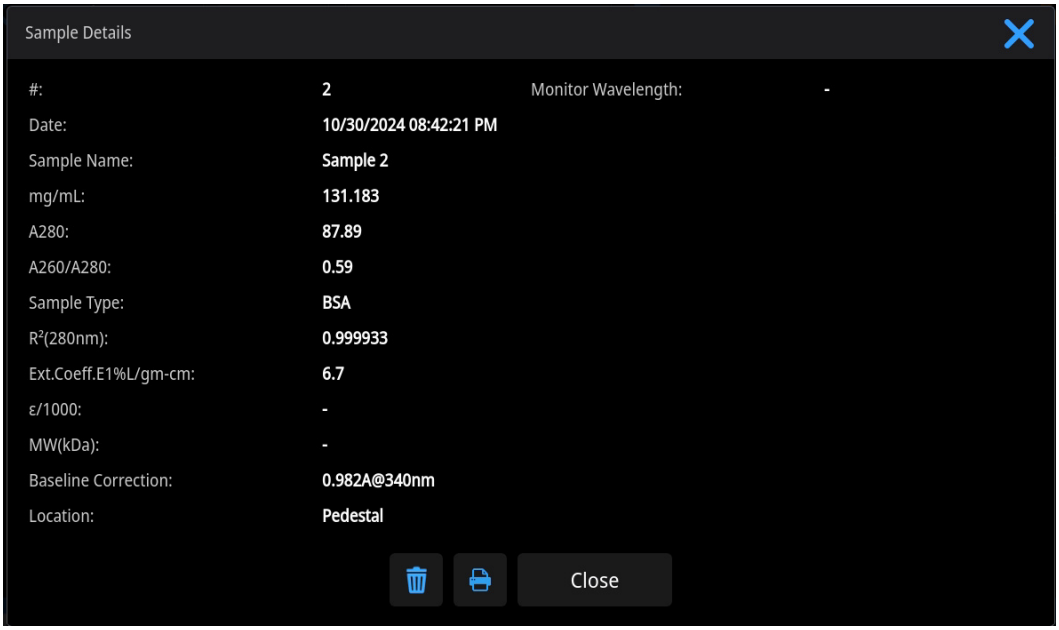
Note Micro-volume absorbance measurements are normalized to a 10.0 mm pathlength equivalent.

Protein A280 Pro measurement screen (PC control)

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

Reported values

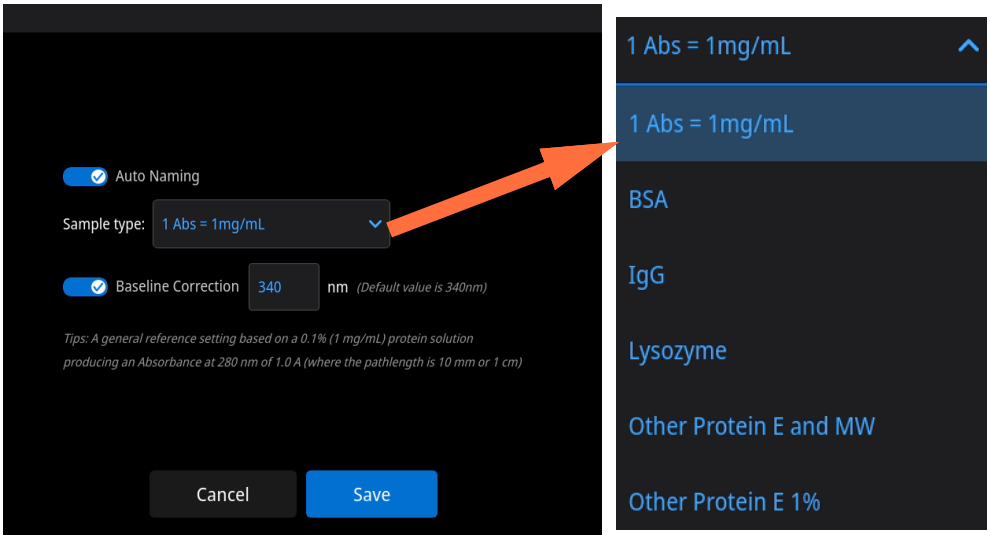
The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



- # (sample number)
- date (date and time sample measurement was taken)
- sample name
- protein concentration
- A280
- A260/A280
- sample type
- R2 (280nm)
- baseline correction
- location
- monitored wavelength
- mass extinction coefficient (1% solution)
- molar extinction coefficient
- molecular weight (kDA)

Settings

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



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

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

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

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Auto Naming	On or off	N/A	When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name.
Baseline Correction	On or off 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.
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 $M^{-1}cm^{-1}$.

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

Detection Limits

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

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

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

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

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

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

Calculations

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

Beer-Lambert Equation (solved for concentration)

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

where:

A = UV absorbance in absorbance units (AU)

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.

Extinction Coefficients for Proteins

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

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

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

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

where:
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

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.

- **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 Ultra software includes the conversion factor when reporting protein concentrations.

Conversions Between g/100 mL and mg/mL

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

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

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

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

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

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

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

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

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

Measured Values

A280 absorbance

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

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

A260 absorbance

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

Reported Values

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

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

- **Sample type**. Determines the extinction coefficient used in conjunction with Beer's Law to calculate sample concentration.
- **R^2 (280nm)**. Coefficient of determination for the line of best fit for 280 nm measurements.
- **Baseline correction**. Wavelength selected for baseline correction and the absorbance detected at that wavelength.
- **Location**. Displays that the measurement was taken from the pedestal.
- **Monitored wavelength**. Enter an additional wavelength whose absorbance value you want included in the report.
- **Mass extinction coefficient (1% solution)**.
- **Molar extinction coefficient**.
- **Molecular weight**.

8

More Applications

Use the NanoDrop Ultra Spectrophotometers and Fluorometers to perform UV-Vis, OD600, Kinetics, or your own Custom measurements.

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

The Custom Methods application provides additional flexibility for users looking for unique information about their samples.

UV-Vis

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

Measure UV-Vis

Reported Results

Settings

Best practices for UV-Vis measurements

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

Measure UV-Vis

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

Before you begin...

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

NOTICE

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

Procedure

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

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

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

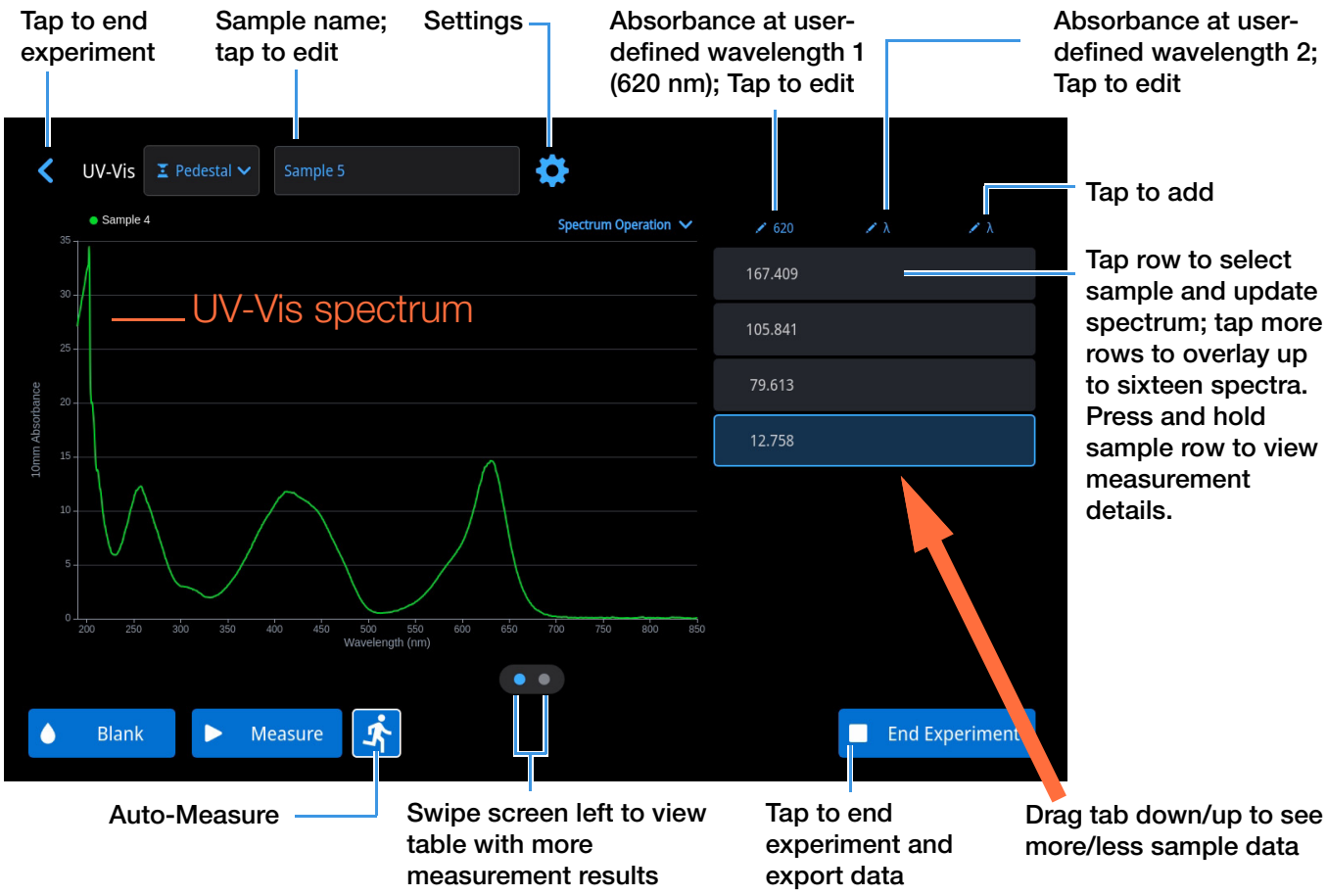
6. Lift the arm and clean both pedestals with a new laboratory wipe or remove the blanking cuvette.
7. Pipette 1-2 µL sample solution onto the pedestal, or insert the sample cuvette into the cuvette holder.
8. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.
   - Cuvette: Select **Measure.**

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

Reported Results

UV-Vis measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example as it appears on the NanoDrop Ultra local control instrument display:



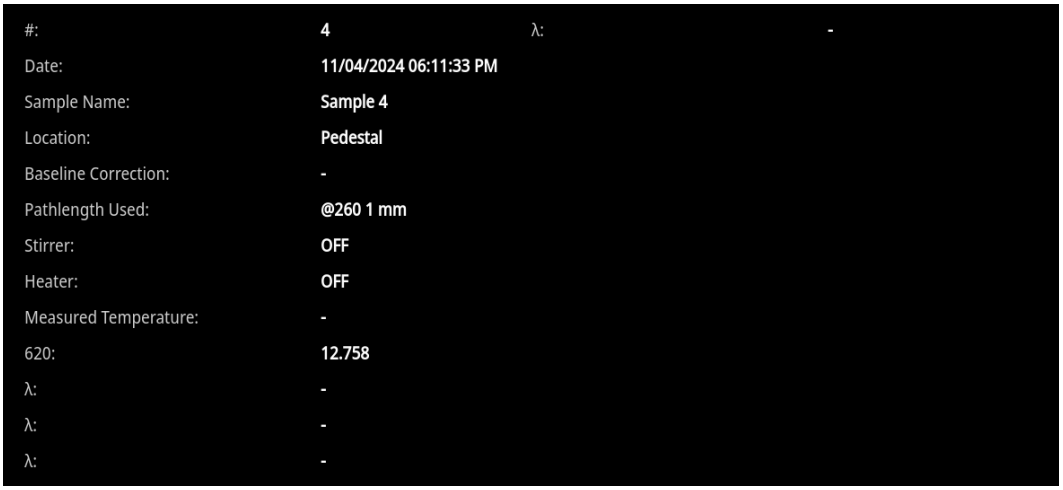
Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

UV-Vis measurement screen (PC control)

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

UV-Vis reported values

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



Settings

The UV-Vis setup screen appears after you select the UV-Vis application from the More Apps tab on the home screen. To show the UV-Vis settings from the UV-Vis measurement screen, select  .

Setting	Available Options	Description
Auto Naming	On or off	When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name.
Monitored wavelengths	Enter up to 40 wavelengths between 190 nm and 850 nm	**User-defined wavelengths to be measured and reported at run time**. Absorbance values for the first three entered wavelengths are displayed in the measurement screen. To see absorbance values for 6 monitored wavelengths, swipe left in the measurement screen to show the Data table. To see all monitored wavelengths, press and hold a sample row to show the Sample Details screen (scroll up to display absorbance values for any additional user-defined wavelengths). **Note**: If Baseline Correction is selected, all displayed absorbance values are the corrected values.
Analytical Wavelength	On or Off Enter analytical wavelength in nm or use default value (220 nm)	This is the wavelength the software will use to determine the pathlength selection. Automated Pathlength cannot be used when Analytical Wavelength is enabled.

Setting	Available Options	Description
Automated Pathlength	On or Off (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 between 220 nm and 850 nm has 10 mm equivalent absorbance value of 12.5 or higher. For wavelengths between 190 nm and 219 nm the change to the shorter pathlength occurs when any wavelength in this range has a 10 mm equivalent absorbance value of 10 or higher. • When deselected, the pedestal pathlength is restricted to 10 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.