

NanoDrop Micro-UV Spectrophotometer

NanoDrop Lite Plus

User Guide

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NanoDrop Lite Plus User Guide

The Thermo Scientific[™] NanoDrop[™] Lite Plus is a small, stand-alone UV spectrophotometer developed specifically for microvolume measurements of nucleic acids and purified proteins. The NanoDrop Lite Plus comes with preloaded software and is designed to be used without a computer. The NanoDrop Lite Plus can also be connected to an accessory printer for printing measurement information on labels.



The sample retention system of the NanoDrop Lite Plus is identical to that used in all NanoDrop instruments: surface tension is used to hold 1-2 μ L of sample between two stainless steel pedestals that contain optical fibers. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

The NanoDrop Lite Plus measures the sample absorption at 230, 260, and 280 nm and corrects for scattering and background effects in the sample with light from a reference wavelength at 340 nm.

General Safety



CAUTION Operating the instrument with the cover off exposes the operator to the instrument's sharp metal edges and delicate fiber optics. Removal of the cover may also void the warranty.

Note The NanoDrop Lite Plus is supplied with a 12 V power supply. Use only the power supply provided with the instrument. The unit also comes with a grounded power cord. Plug this cord into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

The power supply can remain plugged into the NanoDrop Lite Plus while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~3 W at idle, 18 W at working conditions.

NanoDrop Lite Plus Basic Operation

Installation





* For factory calibration only

Menu Selection



Table 1.Navigation menu

Menu option	Function
Back	Return to the previous screen
Home	Return to the Home screen
History	View and export sample history
Settings	View and configure instrument parameters such as display settings, printers, and instrument info.
Maintenance	Perform performance verification, instrument diagnostics and software updates.

Table 2.Sample category reference settings

Nucleic Acids	
dsDNA	factor = 50
ssDNA	factor = 33
RNA	factor = 40
Protein	
Protein (1 Abs= 1 mg/mL)	Default general reference setting
Protein (IgG)	E1%=13.7
Protein (BSA)	E1%=6.7
Lysozyme	E1%=26.4
Other protein (\mathcal{E} + MW)	User entered molar extinction coefficient and molecular weight
Other protein (£1%)	User entered mass extinction coefficient

Settings screen

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Maintenance screen

	Maintenance	
⋒	Reset Sample ID	>
Ë	Performance Verification	>
Q 0	Diagnostics	>
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Measurement Basics

The NanoDrop Lite Plus 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. 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.

Raise the arm and pipette the sample onto the lower pedestal.

1. Lower the arm and initiate a measurement.

The sample column is automatically drawn between the upper and lower pedestals and the measurement is made.

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

Tip Simple wiping with a dry laboratory wipe is sufficient to prevent sample carryover in subsequent measurements.

Nucleic Acid Measurements

Nucleic acid samples can be easily checked for concentration and purity using the NanoDrop Lite Plus spectrophotometer. To measure nucleic acid samples (dsDNA, ssDNA and RNA) from the Home screen, select **Nucleic Acids** and select the appropriate Nucleic Acid application.

Making a Measurement

1. From the **Home** screen, select **Nucleic Acids** and select the appropriate sample type.

For DNA measurements, select either the dsDNA or ssDNA assay.

- 2. Following the on-screen instructions, establish a blank by pipetting 1-2 μ L of the blanking buffer onto the bottom pedestal, lower arm and select **Blank**.
- 3. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
- 4. Measure sample by pipetting 1-2 μ L of sample onto the bottom pedestal, lower arm and select **Measure**.
- 5. Wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

Note Use a fresh aliquot of sample for each measurement.



Nucleic Acid Measurement Screen

Nucleic Acid Calculations

The NanoDrop Lite Plus should be used to measure the concentration of purified nucleic acid samples. Direct nucleic acid concentration measurements assume a purified sample as all absorbance at 260 nm is included in the calculation of the nucleic acid concentration. Carryover of nucleotides, primers, purifying reagents, and cell material into the measured nucleic acid sample will overestimate the nucleic acid concentration.

For nucleic acid quantification, the Beer-Lambert equation is modified to use a conversion factor with units of ng-cm/ μ L. The modified equation used for nucleic acid calculations is the following:

c = (A * CF)/b

where,

- c = the nucleic acid concentration in ng/µL
- **A** = the sample absorbance
- **CF** = the conversion factor in ng-cm/ μ L
- **b** = the pathlength in cm

The generally accepted conversion factors for nucleic acids are:

- Double-stranded DNA: 50 ng-cm/µL
- Single-stranded DNA: 33 ng-cm/µL
- RNA: 40 ng-cm/µL

Table 3. Nucleic acid concentration range

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical reproducibility; based on 10 replicates (SD = ng/μL; CV=%)
dsDNA	2.0 ng/µL	1500 ng/µL	±2.0 ng/μL for sample concentrations between 2.0 and 100 ng/μL samples; ±2% for samples >100 ng/μL
ssDNA	1.3 ng/μL	990 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	1200 ng/µL	±2.0 ng/μL for sample concentrations between 2.0 and 100 ng/μL samples; ±2% for samples >100 ng/μL

Note Absorbance reported is normalized to a 1.0 cm (10.0 mm) pathlength for all measurements.

Protein Measurements

Purified protein samples can be quantified on the NanoDrop Lite Plus spectrophotometer. To measure the concentration of a protein sample, select the **Protein** application from the Home screen and then select the appropriate assay type (1 Abs= 1 mg/mL, IgG, Lysozyme, BSA, or Other Protein).

Making a Measurement

- 1. Select **Protein** from the Home screen.
- 2. Select the appropriate assay type: 1Abs=1mg/mL, IgG, Lysozyme, BSA, or Other Protein.
- 3. Following the on-screen instructions, establish a blank by pipetting 2 µL of the blanking buffer onto the bottom pedestal, lower arm and select **Blank**.
- 4. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
- 5. Confirm Blank by pipetting a fresh aliquot of blanking buffer onto the bottom pedestal, lower the arm and select **Measure**. The resulting absorbance at the analysis wavelength should be no more than 0.04 A. If your absorbance does not meet this criteria, establish a new blank with a fresh aliquot of buffer.
- 6. When measurement is complete, raise the arm and wipe the buffer both the upper and lower pedestals using a dry laboratory wipe.
- 7. Measure sample by pipetting 2 μ L of sample onto the bottom pedestal, lower arm and select **Measure**.
- 8. Wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

Note Use a fresh aliquot of sample for each measurement.

When working with protein samples it is recommended that a 2 μ L sample volume be used to ensure that a liquid column is formed between the upper and lower pedestals. This is especially true for concentrated samples or samples that contain detergents. "Sample Volume Requirements" on page 17.

Protein Measurement Screen



Protein Calculations

Proteins, unlike nucleic acids, exhibit considerable diversity. The Protein A280 application is indicated for purified proteins that contain Trp, Tyr residues or Cys-Cys disulphide bonds and exhibit absorbance at 280 nm. The Protein A280 application measures sample absorbance at 280 nm (A280) and calculates the concentration (mg/mL) using the selected extinction coefficient. The Protein A280 application does not require generation of a standard curve.

The NanoDrop Lite Plus should be used to measure the concentration of purified protein samples. Direct protein concentration measurements assume a highly purified sample as all absorbance at 280 nm is included in the calculation of the protein concentration. Carryover of starting materials, purifying reagents, and cell material into the measured protein sample will result in an artificially high protein concentration.

Protein concentration is calculated using the Beer-Lambert equation:

 $c = A/e^*b$

where,

- **c** = the protein concentration
- **A** = the sample absorbance
- e = the protein specific mass extinction coefficient
- **b** = the pathlength in cm

The protein specific concentration measurement options are:

1 Abs= 1 mg/mL: 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., E1% = 10.

IgG: Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (E) 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⁻¹cm⁻¹.

BSA: Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient (E) 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⁻¹cm⁻¹

Lysozyme: Calculates lysozyme protein concentration using mass extinction coefficient (E) 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⁻¹cm⁻¹ and 39,000 M-1cm⁻¹.

Other protein (E &MW): User entered molar extinction coefficient and molecular weight. Assumes protein has known molar extinction coefficient (E) and molecular weight (MW), where:

(E_{molar})*10=(E_{percent})*(MW_{protein})

Enter MW in kiloDaltons (kDa) and molar extinction coefficient (E) in M⁻¹cm⁻¹ divided by 1000 (i.e., E/1000). For example: For protein with molar extinction coefficient of 210,000 M⁻¹cm⁻¹, enter 210.

Other Protein (E 1%): User entered mass extinction coefficient. Assumes protein has known mass extinction coefficient (E). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (E1%) protein solution.

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical reproducibility (10 replicates, SD = mg/mL; CV=%)
BSA	0.06 mg/mL	45 mg/mL	±0.10 mg/mL (for 0.10-10 mg/mL samples); ±2% for samples >10 mg/mL
lgG	0.03 mg/mL	22 mg/mL	±0.10 mg/mL (for 0.10-10 mg/mL samples); ±2% for samples >10 mg/mL
Lysozyme	0.015 mg/mL	11.4 mg/mL	±0.10 mg/mL (for 0.10-10 mg/mL samples); ±2% for samples >10 mg/mL

	Table	4.Protein	Concentration	Range
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Note Absorbance reported is normalized to a 1.0 cm (10.0 mm) pathlength for all measurements.

Transferring Data From Instrument to Computer

The NanoDrop Lite Plus automatically stores the last 1000 measurements in the internal memory. A USB memory device can be used to transfer these measurements from the NanoDrop Lite Plus to a computer for archival or further analysis.

The internal software of the NanoDrop Lite Plus does not support connecting the NanoDrop Lite Plus to an external hard drive. Only USB memory devices (memory sticks) should be used to transfer data.

Sample data is automatically saved on the instrument.

To transfer data from the instrument to a USB memory device:

- 1. Insert the device.
- 2. From the Home screen, select **History**.
- 3. Select **Export**.

This file is a .csv format and can be transferred to a computer to be opened in Microsoft Excel® or other compatible application.

NOTICE A maximum of 1000 measurements will be saved in the instrument and are available to be transferred to a USB memory device at any time. Measurement #1001 will replace #1.

Helpful Information for Measuring Samples

Sample Volume Requirements

Sample volume is important because it is essential that a proper liquid column is formed and that the path between the upper and lower pedestals is bridged with liquid sample which is held in place by surface tension.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all solutes (including protein, DNA, RNA, buffer salts and detergents) can reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1 μ L volumes are usually sufficient for most sample measurements, increasing the sample size to 2 μ L will ensure proper column formation for samples with reduced surface tension.

This is particularly true when working with protein samples which may contain detergents. Such samples do not "bead" on the pedestal but tend to spread across the measurement surface. To ensure that a liquid column forms in the gap between the upper and lower pedestals, a 2 μ L volume is indicated for such samples.

Protein samples, especially those containing detergents, are subject to bubble formation. Extra care is required while pipetting onto the pedestal to ensure bubbles are not present into the light path of the spectrophotometer.

Field experience indicates that the following volumes are sufficient to ensure reproducibility: Aqueous solutions of nucleic acids: 1 μ L

Aqueous solutions of purified protein: 2 µL

It is best to use a precision pipettor (0-2 μ L) with precision tips to ensure that sufficient sample (1-2 μ L) is delivered. Lower precision pipettors (0-10 μ L and larger) are not as good at delivering 1 μ L volumes to the lower pedestal. If the user is unsure about the sample characteristics or pipettor accuracy, a 2 μ L sample volume is recommended.

Sample Measurement Accuracy and Reproducibility

Sample or aliquot heterogeneity and/or liquid column breakage may result in erroneous or non-reproducible results. Follow the recommendations below to ensure accurate and reproducible results:

• Ensure the pedestal surfaces are clean. A dirty pedestal (i.e., a pedestal with sample dried on it) may cause erroneous absorbance readings (even negative values) and signal saturation. It is good practice to clean the pedestal surfaces with deionized water prior to starting a measurement.

Note Do not use a squirt or spray bottle to apply de-ionized water.

- Use a 1.5-2 µL sample volume. Unexpected results can occur when the liquid sample column is not completely formed during a measurement. During the measurement, visually confirm that the liquid column is formed. Also, proteins and solutions containing surfactants are known to "un-condition" the pedestal surfaces so that the liquid column does not form. If this occurs, use the Thermo Scientific Pedestal Reconditioning compound (PR-1) to recondition the pedestals. Refer to "Reconditioning" on page 30 for more details.
- Heat high molecular weight DNA samples to 63 °C and gently vortex before measurement. Due to the small volumes required by the NanoDrop Lite Plus, it is important to ensure that the sample being measured is homogeneous. Field experience has shown that samples containing large molecules such as genomic or lambda DNA are particularly susceptible to heterogeneity.
- Ensure that a fresh aliquot of blanking solution is used for the blank confirmation prior to making a sample measurement.
- Confirm that blank and sample buffer are the same pH and ionic strength. Some buffer components absorb in the UV range, therefore, it is critical to blank the instrument with the same solution (buffer from the same bottle if possible) in which the sample is suspended.
- Confirm that the sample is not too dilute or too concentrated. Analyzing samples at or near the detection limit will result in variable measurements. Refer to Table 3 and Table 4 for guidance on detection limits.
- Confirm instrument is working within specifications with PV-1. PV-1 is a liquid photometric standard available from Thermo Fisher Scientific and its distributors. It is recommended that the performance verification be performed every six months with a fresh vial of PV-1 to confirm that the instrument is performing within specifications.

Sample Homogeneity

Sampling from heterogeneous solutions, particularly when using small volumes, may result in poor data reproducibility regardless of the technology being used to make the measurement. Genomic DNA, lambda DNA and viscous solutions of highly concentrated nucleic acids or proteins are common examples that require careful attention to ensure homogeneity before sampling. Proteins are subject to denaturation, precipitation, and aggregation and therefore also require special handling to ensure homogeneity before sampling.

Sample Carryover

Wiping of the upper and lower pedestal with a clean, dry laboratory wipe is sufficient to eliminate carryover between samples differing in concentration by as much as three orders of magnitude.

A260/A280 Ratio

Some researchers may encounter a consistent A260/A280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop Lite Plus. The three main causes for this are:

Change in sample acidity - Small changes in solution pH will cause the A260/A280 ratio to vary. Acidic solutions will under-represent the A260/A280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3. When comparing the NanoDrop Lite Plus to other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on the NanoDrop Lite Plus is at the same pH as the diluted sample measured on the second spectrophotometer.

Wavelength Accuracy of the Spectrophotometers - Although the absorbance of a nucleic acid at 260 nm is generally on a plateau, the absorbance curve at 280 nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on A260/A280 ratios. For example, a + 1 nm shift in wavelength accuracy will result in a +0.2 change in the A260/A280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the A260/A280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

The difference in the A260/A280 ratio is important when comparing measurements taken on the NanoDrop Lite Plus to measurements made on other spectrophotometers.

Nucleotide Mix in Your Sample - The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. The following represent the A260/A280 ratios estimated for each nucleotide if measured independently:

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

The resultant A260/A280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the A260/A280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA are "rules of thumb." The actual ratio will depend on the composition of the nucleic acid.

Note RNA will typically have a higher A260/A280 ratio due to the higher ratio of Uracil compared to that of Thymine.

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

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.
- 2. Lift the instrument arm and clean the upper and lower pedestals with a laboratory wipe.
- 3. Measure a water blank:
 - Pipette exactly 1 μL deionized water (DI $H_2O)$ onto the lower pedestal and lower the arm.
 - Tap **Blank** and wait for the measurement to complete.
 - Lift the arm and clean both pedestals with new laboratory wipe.
- 4. Measure the buffer solution:
 - Pipette 1-2 µL buffer solution onto the pedestal, lower the arm, and tap
 Measure
 - Wait for measurement to complete.

The resulting absorbance should vary no more than 0.04A 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" on page 21

- 5. When you are finished with the blanking cycle, tap **End Experiment**.
- 6. Lift the arm and clean both pedestals with a new wipe.

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 Making a Measurement in the application used.

Effect of Evaporation and Solvent Usage

A fresh aliquot of sample should be used for each measurement. Evaporation of the sample during the measurement cycle usually has a minimal effect on absorbance readings and may result in a 1-2% increase in sample concentration. However, repeated measurements on the same sample aliquot will result in increasing concentrations and/or column breakage. Highly volatile solvents, such as hexane, will likely result in evaporation before the measurement can be completed. Less volatile solvents such as DMSO can be used successfully.

Using NanoDrop Lite Plus With Optional Printer



To connect NanoDrop Lite Plus to printer

1. Ensure power supply is disconnected from the NanoDrop Lite Plus.



2. Remove the back cover from the NanoDrop Lite Plus.

3. Firmly push the printer onto the instrument.

The printer will snap into place when properly docked.

Connecting the NanoDrop Lite Plus to the printer



To load paper into printer

4. Make sure the printer is fully docked onto the instrument.

The printer assembly will not work until it is properly docked to the NanoDrop Lite Plus.

- 5. Plug in the power supply and allow NanoDrop Lite Plus to initialize.
- 6. Open the printer cover by pushing the two release buttons at the rear while simultaneously swinging the panel back.
- 7. Unwrap paper roll, insert spool and set the paper roll squarely inside the printer paper roll, in the orientation shown on the icon located on the paper holders.

The paper should be coming out from under the roll toward the feed slot.



8. When you hear the paper feed mechanism move, the printer is ready to load the paper; push the end of the paper into the paper feed slot.

Paper is properly inserted when it engages the print head mechanism and comes out from the slot at the top of the printer.



- 9. Insert the paper roll (with the spool) into the paper roll holder.
- 10. Tap **Feed** from the Printer Settings to advance the paper.

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\$₀		Load Paper:	Feed	
*				

Tip Helpful tips for installing the paper

- Keep the paper centered as it enters the slot on the back of the printer.
- Grab the paper as close to the paper feed slot as possible when feeding into the printer.
- You will hear a noise as the paper engages in the printer. At this time, firmly push the paper into the slot to get it started.
- If none of the above works, clip a small corner from the ends of the paper and reinsert the paper.

Troubleshooting Paper Feed

If the paper does not feed properly (no paper comes out from the top of the printer), remove the paper from the printer and repeat steps 6 through 9.

To remove paper from printer

- 1. Unplug the power supply to printer.
- Open the panel from back of printer by pushing the two release buttons on either side of the paper holders while simultaneously pulling the panel up.
 Once the panel has been removed, you will see a green rocker switch on the right side of the roller.
- 3. Press switch down to release paper (see "Printing Labels" on page 25).
- Pull paper gently out of the printer. Push the green switch up to the horizontal position once paper has been removed.

Note Ensure you return switch to horizontal position before you put the panel on.

- 5. Snap back panel into place.
- 6. To reload paper plug in the power supply to printer and repeat the steps in "To load paper into printer" on page 22.

Printing Labels

When the instrument is connected to the printer, there are three ways to print sample labels:

- If Autoprint is ON, a label will print automatically after each sample measurement.
- To change Autoprint navigate to Home > Settings > Printer. Toggle Autoprint Enabled or Autoprint Disabled.
- When Autoprint is turned off, select Print after measuring a sample.
- If additional labels are required, select Print again.
- Select Print 🖶 when viewing results from previously measured samples in the Sample History screen (Home > History).

This allows labels to be printed from previously measured samples stored in the instrument's memory. Use **Previous** and **Next** to display the sample data to print.



Note The printer labels are cryogenic, and per manufacturing specifications they perform well under a wide temperature range: 70° C to -196° C.

Printer Output

The label will print out the sample name entered on the instrument.

```
#8011 sample
dsDNA 185.3 ηg/UL
260/288:1.87 269/230:2.37 #0011
Feb 23 2022 11:87 ΑΜ
```

Labels can be used on a 1.5 mL tube or in a lab notebook.

The round sticker next to the label fits the top of a 1.5 mL tube and is provided to use as needed for sample identification.

Maintenance

Calibration

All NanoDrop Lite Plus instruments are calibrated at the time of manufacture and include a Factory Test Report. Should recalibration ever become necessary, this procedure must be performed by Thermo Fisher Scientific or the authorized service providers at our dealers. Recalibration is extremely rare and should only be performed when the unit fails the performance verification or when critical optical components are repaired or replaced.

Performance Verification

All NanoDrop Lite Plus instruments are calibrated at the time of manufacture and include a Factory Test Report. It is recommended that a performance verification be performed every six months to verify the instrument is performing within specifications.

An ampoule of Thermo Scientific PV-1 performance verification solution is required to run the performance verification. PV-1 is used to confirm the pathlength accuracy of NanoDrop Lite Plus spectrophotometers.

1. Ensure the pedestals are clean and that a 1 µL water sample "beads" on the lower pedestal, then wipe both pedestals dry.

If water does not bead, recondition the pedestals. See "Reconditioning" on page 30.

- 2. From the Home screen, select Maintenance
- 3. Choose Performance Verification.
- 4. Enter the target absorbance #2 found on the PV-1 vial: Tap the absorbance value field, use the touchscreen keypad to enter the value, and tap **Enter**.

Make sure the target value entered corresponds to the NanoDrop Lite Plus. Target absorbance should be around 1.0.

- 5. Select Run.
- 6. Lift the arm and pipette 1 μ L of deionized water onto the pedestal.
- 7. Close the arm and select **Blank**.
- 8. Wipe the water from the upper and lower pedestals with a laboratory wipe.

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.

- 9. Carefully break the neck of the ampoule to open the PV-1.
- 10. Pipette 1 μ L of PV-1 onto the lower pedestal, close the arm and select **Measure**.

The 1st measurement result will appear on the screen.

11. Wipe the sample from both the upper and lower pedestals with a laboratory wipe, and pipette 1 μ L of PV-1 on the lower pedestal, close the arm and select Measure.

The 2nd measurement result will appear on the screen.

12. Repeat Steps 10 and 11 until 10 measurements have been collected.

Tip To ensure accurate results, be sure to wipe the top and bottom of the pedestal with a dry, lint-free laboratory wipe between measurements. Use a fresh aliquot of PV-1 and a fresh pipette tip for each measurement.

After all 10 replicates have been measured, results will display as Pass/Fail. If the performance verification results indicate Fail, thoroughly clean the pedestal following the directions on the "Pedestal Cleaning and Reconditioning" document or the Cleaning and Reconditioning sections in the User Guide, and then repeat the entire calibration check. If the calibration check results indicate Pass, proceed with step 13.

13. Insert the USB memory device.



14. Select **Export**.

The following message will appear on the screen: Export in progress.....Please do not remove USB drive until export is complete. When this message disappears, it is safe to remove the USB memory device from the NanoDrop Lite Plus.

After the data has been saved to the USB memory device, the data can be transferred to a computer to archive or print. The performance verification is now complete.

The verification report cannot be printed on labels using the optional NanoDrop Lite Plus printer. Only new performance verification reports can be saved on USB memory device and transferred to a computer for printing or archiving.

Only the most recent performance verification is saved to the instrument. Prior performance verification history is overwritten each time a verification is performed. The previous history cannot be retrieved.

To view the previous performance verificaiton

1. Go to the Home screen, select Maintenance.

- 2. Select Performance Verification.
- 3. Select **View Previous**.
- 4. To Export to USB, select Export.

Troubleshooting

- If the instrument does not pass the calibration check using 1 µL aliquots of PV-1, immediately repeat the procedure again using 2 µL aliquots of PV-1.
- If the procedure fails with 1 µL volumes but passes with 2 µL volumes, it is an indication that the pathlengths are within specifications but the pedestals may not be properly conditioned. Clean the pedestals with PR-1 and repeat the calibration check.
- If the instrument still does not pass the performance verification using 2 µL volumes, recalibration is required. Contact technical support. Outside of the US and Canada, please contact your local NanoDrop distributor.

Cleaning

The primary maintenance for the NanoDrop Lite Plus is to keep the pedestal surfaces clean.

- 1. Pipette 3 μ L of deionized water (dH₂O) onto the bottom pedestal.
- 2. Do not use a squirt bottle to apply dH₂O or any other liquid to the surface of the instrument.
- 3. Lower the arm to form a liquid column; let it sit for approximately 2-3 minutes.

Wipe away the water from both the upper and lower pedestals with a dry, lint-free lab wipe.

Tip Between measurements: Wipe the sample from both the upper and lower pedestals with a clean, dry, lint-free lab wipe, to prevent sample carryover and avoid residue buildup.

Tip Between users: A final cleaning of both pedestals with dH_2O is recommended after the last sample measurement is collected.

Additional cleaning: When a more rigorous cleaning protocol is required (i.e. dried proteins) substitute 0.5M HCl for the dH_2O in the procedure above. Follow with 3-5 μ L of dH_2O .

Reconditioning

The pedestal surface may become unconditioned over time, especially when measuring proteins or samples that contain surfactants or detergents. If the surface properties have been compromised, samples may not "bead up" on the pedestal or the liquid column may break during measurement. Use the instrument pedestal reconditioning kit, PR-1, as a rapid means of reconditioning the pedestal back to a condition optimal for liquid column formation.

- 1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
- 2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals and wait 30 seconds for the PR-1 to dry.
- 3. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed.

The appearance of a black residue on the laboratory wipe is normal. Continue wiping the pedestals with a clean lab wipe until the lab wipe shows no black residue.

4. Use canned air to remove excess lint from the diaphragm that surrounds the base of the lower pedestal.

Test the effectiveness of the re-conditioning by pipetting a 1 μ L sample of dH₂O (using a calibrated 2 μ L pipettor) onto the lower pedestal to visually verify that the dH₂O beads up.



The figure on the left shows how an aqueous sample spreads on an unconditioned pedestal. The figure on the right shows how 1 μ L of dH₂O should bead up on a properly conditioned pedestal.

Any buffers containing surfactants or detergent may "un-condition" the measurement pedestal surfaces so that the liquid column does not form well with 1 μ L samples. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. This will "re-condition" the surfaces allowing the liquid sample column to form.

Solvent Compatibility

The NanoDrop Lite Plus spectrophotometer pedestals are compatible with most solvents typically used in life science laboratories. These include:

• acetone	• dilute HNO3	 isopropanol
 acetonitrile 	• dilute acetic acid	methanol
 benzene 	• DMF	 n-propanol
 butanol 	• DMSO	 sodium hydroxide
carbon tetrachloride	 ethanol 	 sodium hypochlorite (bleach)
 chloroform 	• ether	• THF
dilute HCI	 hexane 	• toluene

NOTICE

- All forms of Hydrofluoric Acid (HF) are incompatible, as the fluoride ion will dissolve the quartz fiber optic cable.
- Do not allow alcohol, bleach, acetone or other solvents to remain on the diaphragm surrounding the pedestal for more than a minute as the adhesive keeping the seal in place may be adversely affected. If the seal comes loose, please contact technical support.

Decontamination of Upper and Lower Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions, freshly prepared), may be used to ensure that no biologically active material is present on the upper and lower pedestals.

Note Do not use a squirt or spray bottle to apply the diluted bleach. Always dampen a laboratory wipe with the bleach to clean the top and bottom pedestal surfaces as well as the outside of the instrument. Remove the bleach solution with a lab wipe dampened with water.

Diagnostics

The Diagnostics module is used to confirm the light sources and detectors are working properly.

To perform a diagnostics check:

- 1. Select **Diagnostics** from the Home screen.
- 2. Lift the arm, ensure the pedestals are clean, and lower the arm.
- 3. Select Run.

At the completion of the diagnostics check, values for the parameters listed below will be displayed. A green checkmark is displayed for parameters that PASS, and a red x icon will indicate any FAIL results.

- Integration time
- Detector bias
- Wavelength shift
- Maximum single offset

Troubleshooting

Error Messages

Some error messages are triggered when little to no light reaches the detector during a measurement. If these errors occur, ensure the pedestal surfaces are clean. See "Cleaning" on page 29

Typically dH₂O is sufficient for removal of samples that have dried on the optical fiber pedestals. There are a few cases (i.e., dried proteins) that may require a more rigorous cleaning protocol. For these cases, we recommend that 0.5M HCl be substituted for the 3 μ L of deionized dH₂O. Follow with 3-5 μ L of dH₂O to remove any residual HCl.

Frequently Asked Questions

Q: What are the sample size requirements for the NanoDrop Lite Plus?

A: As little as 1 μ L is usually sufficient for most applications. Generally all additives including protein, DNA, RNA, buffer salts, and detergent-like molecules reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1 μ L volumes are usually sufficient for most sample measurements, increasing the sample size to 2 μ L will ensure proper column formation for samples with reduced surface tension properties. A sample size < 1 μ L is not recommended. See "Sample Volume Requirements" on page 17

Q: What sort of reproducibility and dynamic range for nucleic acids should I expect with the NanoDrop Lite Plus?

A: The dynamic range depends on the assay type. Refer to Table 3 for more details.

Q: What biomolecules can be analyzed with the NanoDrop Lite Plus?

A: The NanoDrop Lite Plus is designed for determining concentrations of purified dsDNA, RNA, ssDNA, and proteins, that absorb at 260 nm or 280 nm.

Q: Do nucleic acids require purification prior to measurement?

A: Yes. Absorbance measurements are not specific for a particular nucleic acid. Any biomolecule that absorbs at 260 nm (DNA, RNA or free nucleotides) will contribute to the total absorbance of the sample.

Q: What happens to sample data if it does not get transferred to a USB device at the time the measurement is made?

A: Data from each measurement is automatically saved in the instrument memory and can be transferred to a memory device at a later time. Samples are identified by the exact time the measurement was made. Data from up to 1000 measurements is stored in the NanoDrop Lite Plus and can be transferred to a PC using the USB device provided with the instrument or another compatible USB device. Once 1000 measurements have been stored in memory, measurement #1001 will be deleted and will be replaced by #1.

Q: Can I quantify proteins using the NanoDrop Lite Plus?

A: Yes. The Protein A280 application can be used for purified proteins. The NanoDrop Lite Plus does not support colorimetric assays.

Q. How do I view previous measurements stored in the instrument?

A. Use Sample History to view or print previous measurements. See "Printing Labels" on page 25.

Q: What is the dynamic (concentration) range and reproducibility for proteins on the NanoDrop Lite Plus?

A: The dynamic range depends on the assay type selected (1 Abs= 1 mg/mL, IgG, BSA, or Other Protein). See Table 4 for more details.

Q: I am using a colorimetric method (e.g., Bradford, BCA, etc.) to determine the protein concentration of my cell extracts. Can I measure the samples using the A280 method on the NanoDrop Lite Plus?

A: No. The Protein A280 application in the NanoDrop Lite Plus is only applicable to purified proteins. Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry are generally used for complex protein samples such as cell lysates and require a standard curve. If you are currently using a colorimetric assay to measure proteins, it is recommended that you use one of the preprogrammed colorimetric methods available on the NanoDrop One/OneC.

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

A: Yes. The highly polished quartz stainless steel surfaces of the sample retention system are resistant to sample adherence. Lint-free lab wipes remove sample very effectively. However, if a sample is left to dry out on the pedestal, more extensive cleaning with water is required. See "Reconditioning" on page 30.

Q: How do I check the accuracy of the NanoDrop Lite Plus?

A: By using the NanoDrop Performance Verification solution to complete a performance verification.

Q: Will the sample size affect the concentration results?

A: No. All calculations are volume independent. Sample concentrations for all applications are calculated using the Beer-Lambert equation, which relates concentration to absorbance using analyte and wavelength specific extinction coefficients or conversion factors.

Q: What light pathlengths are used to make measurements and is the user required to make any calculations relevant to the pathlength?

A: The NanoDrop Lite Plus uses 1.0 mm and 0.2 mm automatically adjusting pathlenghts and all reported concentration results have taken into account the light pathlength. The absorbance reported for all measurements is normalized in a 10 mm pathlength.

Q: What is an appropriate blanking solution?

A: The blanking solution should always be the solvent or buffer used to dissolve the sample (from the same batch or bottle if at all possible), at the same pH and ionic strength.

Q: Why do I have negative absorbance values?

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

Q: How do I keep my sample from spreading on the pedestal?

A: Many protein reagents and buffers contain surfactants that may interfere with the hydrophobic nature of the pedestals, causing samples to spread. Use the NanoDrop PR-1 reconditioning compound as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. PR-1 kits are available through Thermo Fisher Scientific or your local distributor.

Q: Where is the spectrum from my measurement?

A: The NanoDrop Lite Plus does not collect spectral data. It measures absorbance at four wavelengths: 230 nm, 260 nm, 280 nm, and 340 nm for the reference wavelength.

Q. How do I clean the pedestals?

A: See "Cleaning" on page 29. Do not use detergents or isopropanol as cleaning agents as their use may result in the pedestals becoming unconditioned. When the pedestal becomes unconditioned sample droplets will not properly bead on the bottom pedestal.

Q: How often do I need to run Performance Verification?

A: We recommend confirming that the instrument is working within specifications about every 6 months. The Thermo Scientific Performance Verification (PV-1) is required to run the Performance Verification check procedure. PV-1 is available from Thermo Fisher Scientific or one of its authorized distributors.

Q: Does the NanoDrop Lite Plus require a computer to operate?

A: No, the NanoDrop Lite Plus instrument is a standalone unit with local control. Data can be saved to a memory device and transferred to a computer.

Q: How long before I need to replace the light source in the NanoDrop Lite Plus?

A: The xenon flash lamp is expected to last for the lifetime of the instrument.

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

A: The xenon flash lamp is only on during measurements.

Q: Can I connect my computer to the NanoDrop Lite Plus?

A: No. The NanoDrop Lite Plus cannot be connected to a PC. The NanoDrop Lite Plus is a local control instrument running stand-alone software.

Q: Are there solvents that will damage the pedestal measurement surfaces?

A: The NanoDrop Lite Plus measurement pedestals are compatible with most solvents typically used in a life science laboratory including dilute acids as long as they are immediately wiped from the pedestals when the measurement is complete. Hydrofluoric acid (HF), in any form, will damage the pedestal by dissolving the quartz fiber optic cable. Do not use hydrofluoric acid on the pedestal. See "Solvent Compatibility" on page 31.

Warranty

All NanoDrop spectrophotometers and accessories manufactured by Thermo Scientific are under warranty against manufacturing defects in parts and labor for a period of one year. Preventive maintenance, as well as additional one, two, and three year warranty extensions are available. More information about the various plans may be found on our website.