

Thermo Scientific NanoDrop Products

NanoDrop Lite

User Guide

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NanoDrop Lite User Guide

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



The sample retention system of the NanoDrop Lite is identical to that used in all NanoDrop instruments: surface tension is used to hold 1-2 μl of sample between two stainless steel pedestals that contain optical fibers. Using Light Emitting Diode (LED) technology, the NanoDrop Lite measures the sample absorption at 260 and 280 nm and corrects for scattering and background effects in the sample with light from a reference wavelength at 365 nm. The A260 and A280 is calculated with respect to the baseline absorbance at 365 nm. Thus the NanoDrop Lite provides accurate and reproducible measurements for microvolume samples with no need for cuvettes.

When measuring nucleic acids (dsDNA, ssDNA and RNA), the NanoDrop Lite provides concentration information using the A260 measurement and sample purity information using the A260/A280 ratio. When measuring purified protein samples, the NanoDrop Lite provides concentration information using the A280 measurement.

General Safety



NOTICE Be sure that all persons operating this system read the site and safety manual first.



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 is supplied with a 6 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 while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the LED sources are not energized. Since the NanoDrop Lite does not use a power switch, it is recommended that it is positioned in a fashion that allows easy access to the power connection so that it can be easily disconnected.

NanoDrop Lite Basic Operation

Installation

Figure 1. Back view

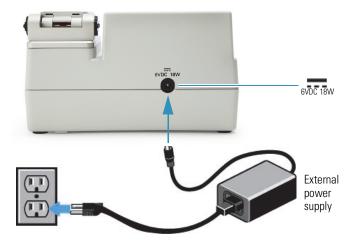
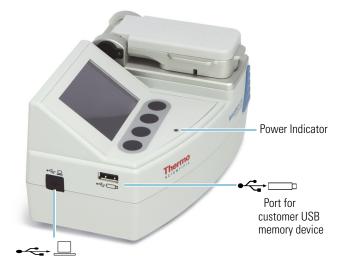


Figure 2. Left side view



For factory use only

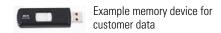
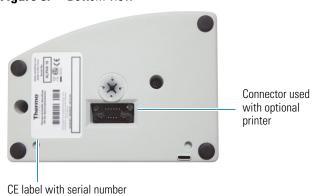
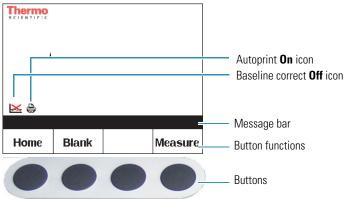


Figure 3. Bottom view



Keypad Operation

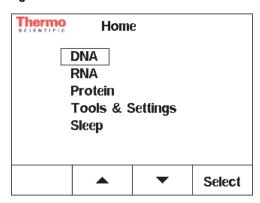
Figure 4. Basic screen features



Key or button	Function	
	 When pressed, initiates a specific function as displayed above each button Functions will change depending on the screen 	
_++++	•Arrow keys that control the direction of the cursor	
Select	•Accepts entered or selected values •Advances to the next parameter or screen	
Home	•Returns to the main menu	
Blank	•Initiates blank measurement	
Measure	•Initiates sample measurement	
Change	•Allows user to change settings	
Back	•Returns to previous screen	

Menu Selection

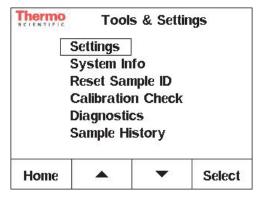
Figure 5. Home screen



DNA	
dsDNA	factor = 50
ssDNA	factor = 33
RNA	
RNA	factor = 40
Protein	
Protein (1A/cm = 1 mg/ml)	Default general reference setting
Protein (IgG)	E1%=13.7
Protein (BSA)	E1%=6.7

Tools & Settings Function

Figure 6. Tools & Settings screen



Settings	
Autoprint: On/Off	Off is the default state. When Autoprint On is selected, and the NanoDrop Lite is docked onto the printer, a label will print automatically every time a measurement is made. Labels will not be printed automatically if Autoprint Off is selected.
Baseline correct: On/Off	 On is the default state. It is best to keep Baseline correct setting On. If Baseline correct setting is Off, the instrument no longer uses the 365 nm correction.
Date & Time	 Set at the factory for Eastern Standard Time, USA. Upon instrument installation, ensure the time and date is set for the local time zone.

Tools & Settings	Function	
System Info		
Product	•NanoDrop Lite	
Serial Number	•Instrument identification.	
Firmware Version	•Version number of firmware currently installed.	
Cal. Check Date	 Date shown is the last date the calibration was checked on the NanoDrop Lite. 	
Reset Sample ID		
Reset Sample ID? Yes/No	•When Yes is selected, the instrument will reset sample ID number back to #1.	
Calibration Check		
	 Verifies that the pathlengths are within tolerance. 	
	•It is recommended that a pedestal calibration check be performed every six months to verify that the instrument is performing within specifications.	
New Cal. Check	•Follow on-screen directions to perform new calibration check using the CF-1 Calibration Check solution.	
	•See NanoDrop Lite Calibration Check Procedure for more details.	
View Previous Cal. Check	•Displays data from last calibration check.	
Diagnostics		
	•Follow on-screen directions. Test checks the LED output and ensures that arm sensor and pedestals are operating correctly. •Results will be Pass/Fail.	
Sample History		
	 Displays previous measurements Print option available. The instrument memory holds data for 500 samples. Sample data #501 overrides sample data #1. 	

Sleep	Function
	 When Sleep is selected, the instrument enters a low-power state.
	•The screen will go black and the blue LED below the keypad will pulse.
	 To wake the instrument, push any button or raise the arm.

Measurement Basics

A 1-2 μ l sample is pipetted directly onto the lower pedestal. A fiber optic cable (the source fiber) is embedded within the lower pedestal while a receiving fiber is imbedded in the upper pedestal and the arm. Light emitted from the LEDs traverses the sample and absorbance is measured using a silicon photodiode.

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. The formation of the liquid column during measurement may be viewed through the aperture on the side of the NanoDrop Lite arm.
- 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 spectrophotometer. To measure nucleic acid samples (dsDNA, ssDNA and RNA) select the appropriate Nucleic Acid application from the **Home** screen.

Making a Measurement

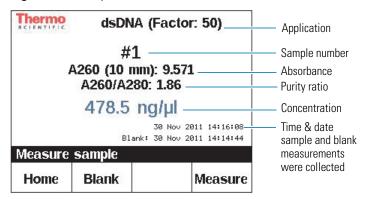
- Select the appropriate application from the Home screen (DNA or RNA).
 For DNA measurements, select either the dsDNA or ssDNA assay.
- 2. Following the on-screen instructions, establish a blank by pipetting 1-2 μ l of the blanking buffer onto the bottom pedestal, lower arm and press **Blank**.
- 3. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.

- 4. Confirm Blank by pipetting a fresh aliquot of blanking buffer onto the bottom pedestal, lower the arm and press **Blank**.
- 5. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
- 6. Measure sample by pipetting 1-2 μl of sample onto the bottom pedestal, lower arm and press Measure.
- 7. 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

Figure 7. Sample measurement screen



Nucleic Acid Calculations

The NanoDrop Lite 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)/1

where,

c = the nucleic acid concentration in ng/μl

A = the sample absorbance

CF = the conversion factor in ng-cm/ μ l

1 = 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 1. Nucleic acid concentration range

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical reproducibility (10 replicates, SD = ng/μl; CV=%)
dsDNA	4.0 ng/μl	1500 ng/μl	4.0 - 100 ng/μl: +/- 2 ng/μl >100 ng/μl: +/- 2%
ssDNA	2.6 ng/μl	990 ng/μl	2.6 - 66 ng/μl: +/- 1.3 ng/μl >66 ng/μl: +/- 2%
RNA	3.2 ng/μl	1200 ng/μl	3.2 - 80 ng/μl: +/- 1.6 ng/μl >80 ng/μl: +/- 2%

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 spectrophotometer. To measure the concentration of a protein sample, select the **Protein** application from the **Home** screen and then select the appropriate assay type (1A/cm = 1 mg/ml, IgG or BSA).

Making a Measurement

- 1. Select Protein from the Home screen.
- 2. Select the appropriate assay type: 1A/cm=1mg/ml, IgG, or BSA.
- 3. Following the on-screen instructions, establish a blank by pipetting 2 μ l of the blanking buffer onto the bottom pedestal, lower arm and press **Blank**.

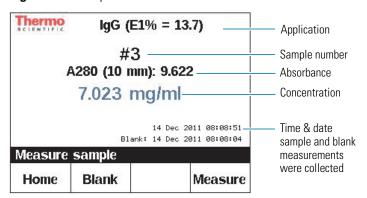
- 4. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
- 5. Confirm Blank by pipetting a fresh aliquot of blanking buffer onto the bottom pedestal, lower the arm and press **Blank**.
- 6. When measurement is complete, raise the arm and wipe the buffer both the upper and lower pedestals using a dry laboratory wipe.
- 7. Measure sample by pipetting 2 μl of sample onto the bottom pedestal, lower arm and press **Measure**.
- 8. Wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

Note A fresh aliquot of sample should be used 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. See "Sample Volume Requirements" on page 13.

Protein Measurement Screen

Figure 8. Sample 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 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:

 $c = A/\epsilon^* l$

where,

c = the protein concentration

A = the sample absorbance

ε = the protein specific mass extinction coefficient

1 = the pathlength in cm

The protein specific concentration measurement options are:

1A/cm = 1 mg/ml: A general reference setting based on a 0.1% (1mg/ml) protein solution producing an Absorbance at 280 nm of 1.0A (where the pathlength is 10 mm or 1 cm). This is the default setting for the **Protein** application.

IgG: Immunoglobulin G reference. Sample protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.

BSA: Bovine Serum Albumin reference. Sample protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution.

Table 2. Protein Concentration Range

Extinction Coefficient	Lower Detection Limit	Upper Detection Limit	Typical reproducibility (10 replicates, SD = mg/ml; CV=%)
1Abs=1 mg/ml	0.08 mg/ml	30 mg/ml	0.08 - 2 mg/ml: +/- 0.04 mg/ml >2 mg/ml: +/- 2%
BSA	0.12 mg/ml	45 mg/ml	0.12 - 3 mg/ml: +/-0.06 mg/ml >3 mg/ml: +/-2%
IgG	0.06 mg/ml	21 mg/ml	0.06 - 1.4 mg/ml:+/-0.03 mg/ml >1.4 mg/ml:+/-2%

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

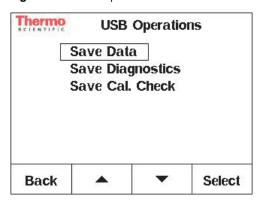
Transferring Data From Instrument to Computer

The NanoDrop Lite automatically stores the last 500 measurements in the internal memory. Measurements include samples, blanks, and blank confirmations. A USB memory device can be used to transfer these measurements from the NanoDrop Lite to a computer for archival or further analysis.

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

These options appear when a USB device is inserted:

Figure 9. USB Operations

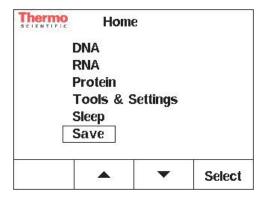


USB Operations	Function
Save Data	Saves all data currently in instrument's memory to USB memory device.
Save Diagnostics	Appends diagnostic data to sample measurements and saves to USB memory device.
Save Cal. Check	Saves the last calibration check data to USB memory device.

The sample data is automatically saved on the instrument. To transfer data from the instrument to a USB memory device, insert the device and select **Save Data** from the **USB Operations** menu. This file can be transferred to a computer and opened in Microsoft Excel*. The **Save Data** option will only appear when a USB device is inserted.

If a USB memory device is inserted, the Save option appears on the Home screen.

Figure 10. Save option from Home screen



Use the **Save** option on the **Home** screen to return to the USB operations menu shown in Figure 9.

NOTICE A maximum of 500 measurements will be saved in the instrument and are available to be transferred to a USB memory device at any time. Measurement #501 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 $2 \mu l$ 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 μl 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. Although the second blank measurement is designed to prevent "false blanks" that are due to dried sample adhering to pedestals, 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 25 for more details.
- Heat high molecular weight DNA samples to 55 °C and gently vortex before
 measurement. Due to the small volumes required by the NanoDrop Lite, 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 (the second blank) 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 1 and Table 2 for guidance on detection limits.

Confirm instrument is working within specifications with CF-1. CF-1 is a concentrated
potassium dichromate calibration standard available from Thermo Fisher Scientific and
its distributors. It is recommended that the calibration check procedure be performed
every six months with a fresh vial of CF-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. The three main causes for this are:

Change in sample acidity - Small changes in solution pH will cause the A260/A280 ratio to vary¹. 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 to other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on the NanoDrop Lite 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.

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

The difference in the A260/A280 ratio is important when comparing measurements taken on the NanoDrop Lite to measurements made on other spectrophotometers. The NanoDrop Lite uses LED sources centered at 260 and 280 nm. The selection of quality LEDs and narrow band filters combine to ensure the highest wavelength accuracy. Therefore, the NanoDrop Lite has no moving parts and wavelength calibration is not required.

Nucleotide Mix in Your Sample - The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios². 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.

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 With Optional Printer

To connect NanoDrop Lite to printer

- 1. Ensure power supply is disconnected from both the NanoDrop™ Lite and the printer.
- 2. Firmly push the instrument onto the printer base as shown.

The instrument will snap into place when properly docked. The NanoDrop Lite is now powered through the printer.

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² Leninger, A. L. Biochemistry, 2nd ed., Worth Publishers, New York, 1975

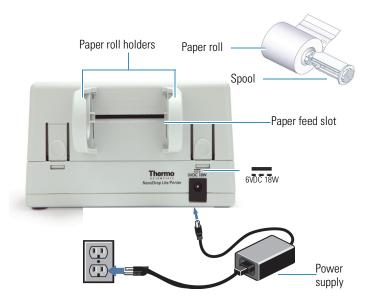
Figure 11. Connecting the NanoDrop Lite to the printer



To load paper into printer

- Make sure the instrument is fully docked onto the printer.The printer assembly will not work until the NanoDrop Lite is properly docked.
- 4. Plug in the power supply to printer and allow NanoDrop Lite to initialize.

Figure 12. Back view



5. Unwrap paper roll, insert spool and set the paper roll squarely behind the printer, in the orientation shown on the icon located on the side of the paper holders.

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

Figure 13. Paper holder



6. When you hear the paper feed mechanism move and the Home screen appears on the NanoDrop Lite, the printer is ready to load the paper; push the end of the paper into the paper feed slot on the printer back cover.

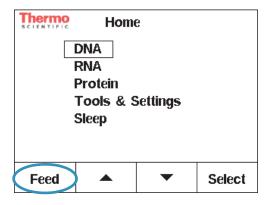
Paper is properly inserted when it engages the print head mechanism and comes out from the slot at the top of the printer.

Figure 14. Inserting paper



- 7. Insert the paper roll (with the spool) into the paper roll holder.
- 8. Press Feed from the Home screen to advance the paper.

Figure 15. The Feed option appears on the Home screen when NanoDrop Lite is docked on the printer



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.

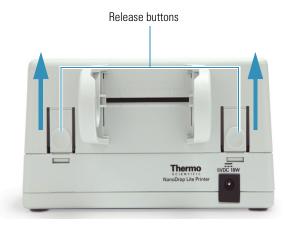
Troubleshooting Paper Feed

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 3 through 6.

❖ To remove paper from printer

- 1. Unplug the power supply to printer.
- 2. Remove panel from back of printer by pushing the two release buttons on either side of the paper holders while simultaneously pulling the panel up.

Figure 16. Release button location



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).
- 4. Pull paper gently out of the printer.

Figure 17. Rocker switch location



5. Push the green switch up to the horizontal position once paper has been removed.

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

- 6. Snap back panel into place.
- 7. To reload paper plug in the power supply to printer and repeat the steps in "To load paper into printer" section.

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 > Tools & Settings > Settings. Press Change to
- toggle to Autoprint On or Autoprint Off.

 When Autoprint is turned off press Print after measuring a sample
- When Autoprint is turned off, press Print after measuring a sample.
 If additional labels are required, press Print again.
- Press Print when viewing results from previously measured samples in the Sample History screen (Home > Tools & Settings > Sample History).

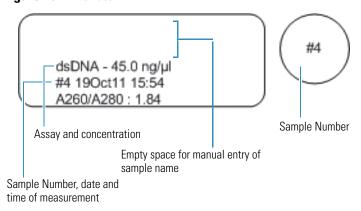
This allows labels to be printed from previously measured samples stored in the instrument's memory. Use the arrow keys to display the sample data to print.

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 printed labels provide space above the data for writing the sample name.

Figure 18. Printer label



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.

Printer Serial Number Location

The printer serial number is located on the label on the bottom of your printer. You will need this number to identify your printer to Technical Support and service.

Figure 19. Serial number



Maintenance

Calibration

All NanoDrop Lite 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 calibration check procedure or when critical optical components are repaired or replaced.

Calibration Check

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

A vial of Thermo Scientific CF-1 Calibration Fluid is required to run the calibration check procedure. CF-1 is an aqueous potassium dichromate $(K_2Cr_2O_7)$ solution used to confirm the pathlength accuracy of NanoDrop Lite spectrophotometers.

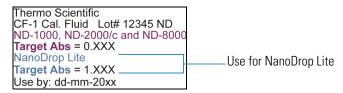
1. Ensure the pedestals are clean and that a 1 μ l water sample "beads" on the lower pedestal, then wipe both pedestals dry.

If water does not bead, clean pedestals using the PR-1 kit.

- 2. From the Home screen, choose Tools & Settings and press Select.
- 3. Choose Calibration Check and press Select.
- 4. Choose New Cal. Check and press Select.
- 5. Enter the **Target Abs** found on the CF-1 vial by using the up or down arrows and press **Next**.

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

Figure 20. Example of CF-1 label



- 6. Lift the arm and pipette 1 µl of deionized water onto the pedestal.
- 7. Close the arm and press Blank.
- 8. Wipe the water from the upper and lower pedestals with a laboratory wipe.

- 9. Repeat Steps 6 and 7 to confirm Blank using a fresh aliquot of deionized water.
- 10. Wipe the water from the upper and lower pedestals with a laboratory wipe.
- 11. Before opening the ampoule of CF-1, shake vigorously to ensure solution is thoroughly mixed and tap lightly to ensure solution is collected in the bottom portion of the ampoule.
- 12. Carefully break the neck of the ampoule to open the CF-1.

Note CF-1 is supplied in a single-use vial. CF-1 must be used within one hour of opening the vial. Exposure to the environment or transferring the fluid to another container may result in significant change in concentration.

- 13. Pipette 1 µl of CF-1 onto the lower pedestal, close the arm and press Measure.
 - The 1st measurement result will appear on the screen.
- 14. Wipe the sample from both the upper and lower pedestals with a laboratory wipe, and pipette 1 µl of CF-1 on the lower pedestal, close the arm and press **Measure**.
 - The 2nd measurement result will appear on the screen.
- 15. Repeat Steps 13 and 14 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 CF-1 and a fresh pipette tip for each measurement.

After all 10 replicates have been measured, results will display as Pass/Fail. If the calibration check 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 16.

- 16. Insert the USB memory device.
- 17. Choose Save Cal. Check and press Select.

If the memory device was in place at the start of the calibration check procedure, go to the Home screen, choose Save, press Select, choose Save Cal. Check, and press Select.

The following message will appear on the screen: Saving.....Do not remove media. When this message disappears, it is safe to remove the USB memory device from the NanoDrop Lite.

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

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

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

❖ To view the previous calibration check

- 1. Go to the Home screen, choose Tools & Settings and press Select.
- 2. Choose Calibration Check and press Select.
- 3. Choose View Previous Cal. Check and press Select.

Note The results of the last calibration check performed are available for viewing on the instrument screen, but cannot be printed or saved to a USB memory device.

Troubleshooting

- If the instrument does not pass the calibration check using 1 μl aliquots of CF-1, immediately repeat the procedure again using 2 μl aliquots of CF-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 calibration check 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 is to keep the pedestal surfaces clean.

- 1. Pipette 3 μ l of deionized water (dH₂O) onto the bottom pedestal. Do not use a squirt bottle to apply dH₂O or any other liquid to the surface of the instrument.
- 2. Lower the arm to form a liquid column; let it sit for approximately 2-3 minutes.
- 3. Wipe away the water from both the upper and lower pedestals with a dry, lint-free lab wipe.

Tin

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.

Between users: A final cleaning of both pedestals with dH₂O 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 spectrophotometer pedestals are compatible with most solvents typically used in life science laboratories. These include:

- acetone
- acetonitrile
- benzene
- butanol
- carbon tetrachloride
- chloroform
- dilute HCl

- dilute HNO₃
- dilute acetic acid
- DMF
- DMSO
- ethanol
- ether
- hexane

- isopropanol
- methanol
- n-propanol
- sodium hydroxide
- sodium hypochlorite (bleach)
- THF
- 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 **Tools & Settings** page.
- 2. Lift the arm and ensure the pedestals are clean.

3. Add 1 µl of deionized water to the lower pedestal and lower the arm.

At the completion of the diagnostics check, a PASS or FAIL result will be displayed for each item tested.

Troubleshooting

Error Messages

Some error messages (e.g., 802, 804, 806 and 808) are triggered when little to no light reaches the detector during a measurement. If these errors occur, ensure the pedestal surfaces are clean. Refer to "Cleaning" on page 24

Typically dH_2O 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 dH_2O . Follow with 3-5 μ l of dH_2O to remove any residual HCl.

Frequently Asked Questions

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

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 13

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

A: The dynamic range depends on the assay type. Refer to Table 1 for more details.

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

A: The NanoDrop Lite 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 500 measurements is stored in the NanoDrop Lite and can be transferred to a PC using the USB device provided with the instrument or another compatible USB device. Once 500 measurements have been stored in memory, measurement #501 will be deleted and will be replaced by #1.

Q: Can I quantify proteins using the NanoDrop Lite?

A: Yes. The Protein A280 application can be used for purified proteins. The NanoDrop Lite does not support colorimetric assays and does not provide A260/A280 ratios for proteins.

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 20.

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

A: The dynamic range depends on the assay type selected (1A/cm = 1 mg/ml, IgG or BSA). See Table 2 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?

A: No. The Protein A280 application in the NanoDrop Lite 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 2000/2000c.

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 25.

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

A: By using the NanoDrop Calibration Check Fluid to perform a calibration check. CF-1 is prepared from the NIST potassium dichromate standard SRM935 in acidified reagent grade water.

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 uses a 0.5 mm light pathlength 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 does not collect spectral data. It measures absorbance at three different wavelengths: a reference wavelength, 260 nm and 280 nm.

Q. How do I clean the pedestals?

A: See "Cleaning" on page 24. 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 check the calibration?

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

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

A: No, the NanoDrop Lite 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?

A: The LEDs are expected to last for the lifetime of the instrument.

Q: Are the LEDs continuously on, or are they on only when performing a measurement?

A: The LEDs are only on during measurements.

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

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

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

A: The NanoDrop Lite 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. Only 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 26.

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.