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Nanodrop Lite (Shared Equipment Lab) V.1

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We use this protocol and it's working

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Abstract

How to use the Nanodrop Lite.

Guidelines

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.

Before start

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.



Basic Operation

- 1 Open arm or push any button to wake instrument.
Using the bottle of deionized water, slightly wet a Kimwipe and clean the lower and upper pedestal (metal prongs).

Note

Although 1 μL volumes are usually sufficient for most sample measurements, increasing the sample size to  2 μL 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 μL volume is indicated for such samples.

- 2 Select measurement type:

STEP CASE

Nucleic Acids 10 steps

Nucleic Acid Measurements

- 3 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.
- 4 Select the appropriate application from the Home screen (DNA or RNA). For DNA measurements, select either the **dsDNA** or **ssDNA** assay.
- 5 Following the on-screen instructions, establish a blank by pipetting  2 μL of the blanking buffer (RO or elution solution your DNA is in) onto the bottom pedestal, lower arm and press **Blank**.



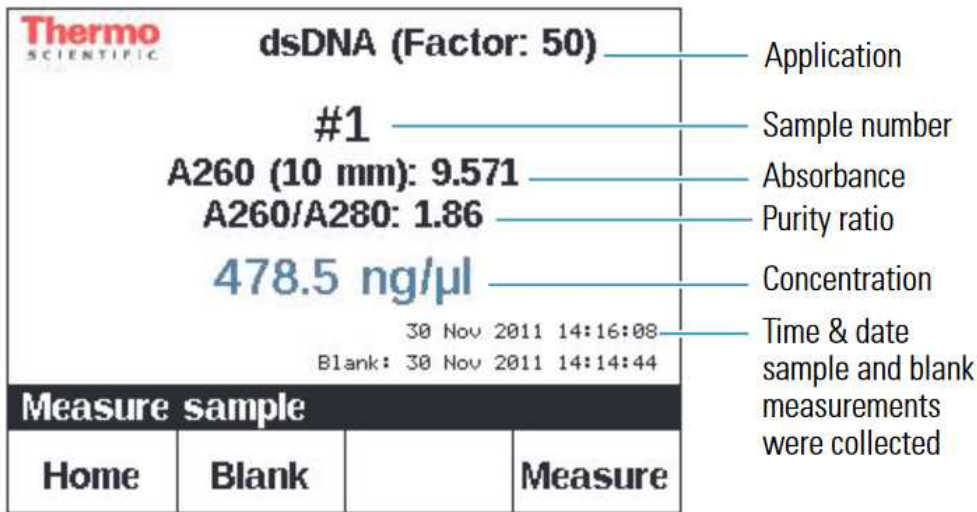
Note

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.

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

Note

Confirm that the sample is not too dilute or too concentrated. Analyzing samples at or near the detection limit will result in variable measurements.



Sample measurement screen

- 8.1 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.

Nucleic Acid Calculations

- 9 Nucleic acid concentration ranges:

	A	B	C
	Sample Type Lower	Lower Detection Limit	Upper Detection Limit
	dsDNA	4.0 ng/ul	1500 ng/ul
	ssDNA	2.6 ng/ul	990 ng/ul
	RNA	3.2 ng/ul	1200 ng/ul



Note

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.

Protocol references

<https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FCAD%2Fmanuals%2FUser-Guide-2752-NanoDrop-Lite-UG.pdf>