

Thermo Scientific NanoDrop 2000 Spectrophotometer

1. Login the computer with your credentials
2. Open the “NanoDrop 2000” software. The NanoDrop will then turn on.
3. From the menu, select the desired type of measurement
4. Ensure the measurement arm is down and allow the NanoDrop to calibrate
5. Lift the measurement arm and with a pipette, load in a blank solution (using the quantities in the references). Lower the measurement arm and press the “Blank” button in the top left corner of the software
6. After the instrument is done measuring, lift the measurement arm and clean the blank off the upper and lower measurement stage with a lint-free wipe
7. With a pipette, load the sample onto the measurement stage (using the quantities in the references below as a guide) and lower the measurement arm. Press the “Measure” button in the upper left hand corner to conduct measurement.
8. Clean the sample off the upper and lower measurement stage with a lint-free wipe

References (Field experience indicates that the following volumes are sufficient to ensure reproducibility)

Aqueous solutions of nucleic acids: 1 μL

Purified protein: 2 μL

Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2 μL

Microbial cell suspensions: 2 μL

From the Manual

Sample Retention Pedestal Measurements

A 1-2 μL sample is pipetted onto a measurement pedestal. A smaller, 0.5 μL volume sample, may be used for concentrated nucleic acid and protein A280 samples. A fiber optic cable (the receiving fiber) is embedded within this pedestal. A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the ends of the two fibers. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array analyzes the light passing through the sample. The instrument is controlled by PC based software, and the data is stored in workbook files (*.twbk) on the PC.

Pedestal Sample Size Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the pathlength between the upper and lower measurement pedestals is bridged with sample. The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives (including protein, DNA, RNA, buffer salts and detergent-like molecules) can reduce the surface tension by interfering with the hydrogen bonding between water molecules. 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.

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