Measuring nucleic acid/protein concentration with NanoDrop 1000 v3.6 spectrophotometer

Phizicky lab (3.7524) user: Nano Drop password: nano01	Wedekind lab (3-7418) located in the lab w/o windows, next to Kielkopf imager room No user/password

Along with samples bring pipettor, tips, nuclease-free water, and paper to record results.
For all steps load 2 µl* onto ***lower pedestal***, ensuring no bubbles, and lower sampling arm to "down" position. Rub the pedestal with clean kimwipe to dry between samples.

1. Wipe sample pedestal (always both upper and lower parts) with kimwipe.
2. Start program: ND-1000 V3.6.0 and select appropriate sample type (see below).
3. Close the lid before starting the initialization, which is a wavelength check. Initialize dry. Be sure to wipe the pedestal first.
4. Add a drop of your buffer to the pedestal of the same volume as your sample – 2 uL for proteins, 1 uL might be OK for nucleic acid but 2 uL strongly recommended by manufacturer. *
5. "Blank" instrument with appropriate buffer/solvent at same volume as used for your sample.
6. When finished, wipe blanking buffer from pedestals.
7. Reanalyze an aliquot of blanking buffer as though it was a sample by loading then clicking "Measure". Spectrum should be flat line.
8. Select appropriate program to measure your sample type (see below). For "Protein A280", select suboption "Other Protein" and enter your extinction coefficient & MW. Also record the A280/A260 for future double checks of concentration and A280:A260 ratio.
9. "Measure" sample concentrations, wiping pedestal with kimwipe between samples. Be sure there are no air bubbles.
10. Check that the spectra make sense. Protein peaks are A280/A230. RNA at A260.
11. Repeat measurement 3-5 times (5 times for ±0.1 mg/mL error in range 0.1 – 10 mg/mL, 2% error for >10 mg/mL up to 100 mg/mL (concentration most proteins).
12. Consider whether you are in a reliable absorbance range. Nanodrop works best at 0.5 – 1 AU. If you are 10x above or below this, error is high.
13. If you fail to record a sample value before the next one is displayed, click "show report" to see all samples from your current run. The default is to "start report" and record sample values automatically.
14. Spectra images can be saved as JPG by selecting "Save Window" from "File" pull-down menu.
15. When all samples finished, clean pedestal with N-free water and wipe dry.
16. Sometimes proteins or DMSO/detergent will "un-condition" the pedestal such that a column no longer forms. Rub aggressively 30-40x (or buy PR-1 reconditioning compound).

For nucleic acids:

Further select DNA/RNA as appropriate from drop-down menu in window upper right.

Measure Abs at 260 nm. For pure nucleic acids in water, should see 1 peak at 260.

1 Abs unit at 260 for dsDNA=50 ng/µL
RNA concentration should be calculated from extinction coefficient (supplied by oligo manufacturer):
A260/Extinction=Concentration (M)
Accurately measures up to 3700 ng/ µL nucleic acid concentration

Purity may be estimated from absorbance ratio 260/280:
DNA: ~1.8-2.0
RNA: ~2.0-2.3

For proteins:

Select Protein A280 option. A sample type can be selected. If you have your extinction coefficient and/or MW handy, use Other protein (E & MW) or E1%. Otherwise, "1 Abs = 1 mg/mL" will give an estimated concentration in mg/mL for this typical protein extinction coefficient.
Measure Abs at 280 nm. Should see two peaks—one at 280, larger ~230 (peptide bond).

A280/Extinction**=Concentration (M)

(concentration(M)•MW(kD))•1000=mg/mL

purity (260/280): ~0.5-0.6

*while aqueous nucleic acids may be measured from 1 µl if necessary, 2 µl is always needed for protein samples (due to lower surface tension). Manual recommends 2 uL.
**extinction coefficient and MW may be calculated from amino acid sequence at http://web.expasy.org/protparam

Normalization: Nucleic Acid and Protein A280 options automatically sets baseline to the absorbance at 340 nm when the "Normalization" box is checked (default). This is fine for most samples that lack a fluorophore. Uncheck Normalization and rerun to confirm value is similar for fluorescein-labeled oligos.

Path length: Nucleic Acid and Protein A280 options automatically switch between 0.1 and 0.2 path lengths at low/high macromolecule concentrations depending on the absorbance of the solution. The program automatically corrects the displayed/saved absorbance values to the equivalent of the 10 mm (1 cm) path length.

Other, e.g. Fluorophores

Select UV-Vis scan.
Scan range limits are 220 – 750 nm
Use 2 uL for samples containing DMSO

Normalization: UV-Vis normalization option automatically sets baseline to the minimum absorbance value between 400 and 700 nm from the entire spectrum, which should be fine for most samples.

Baseline: The horizontal bar can be dragged to set a new baseline. The absorbance is displayed in the baseline box to the right and subtracted from the whole spectrum (not advised unless weird minima between 400 and 700 are present).

Max Absorbance: Cosmetic, used to scale upper limit of y-axis

HiAbs: Use for measuring samples with high absorbance.
Physically uses 0.2 mm path normalized to 0.1 mm, which reduces the absorbance for concentrated samples. Displayed as red line overlaid with black spectrum from traditional 1 mm path length. (guess these are the same spectra but scaled for path length? Unclear).
Normally you would not want to use HiAbs option.