Microvolume Spectrophotometry

Microvolume spectrophotometers (heretofore "Nanodrop") allow the absorbance spectrum to be measured from a mere 1–2 µL of sample pipetted onto a pedestal, compared to 0.5–1 mL needed for cuvette measurements. This can provide an estimate of DNA/RNA concentration good enough for assessing purity and concentration for DNA assemblies. TECAN plate readers have an accessory *NanoQuant Plate* that performs the same function for DNA analysis, but has sixteen quartz lens wells for the samples for higher throughput.
NEB: A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers

A Nanodrop can also provide an estimate of cell concentration when tracking the growth of a culture, so that a larger, accurate cuvette measurement can be made only when the cell density is estimated to be within the desired range, *e.g.* log phase.

Nucleic Acids and Proteins

1. **Pre-Clean:** With a MilliQ water squirt bottle, wet the tip of a low-lint lab tissue wipe (*e.g.* KimWipe) and wipe the Nanodrop /NanoQuant pedestal (base and top), the surfaces that contact the sample. Then wipe dry.
2. **Initialize:** Select the software program, *e.g.* Nucleic Acids or Protein
3. **Blank:** Load 1–2 µL of the solvent blank, *e.g.* water or Tris buffer for DNA.
   Click *Blank* on the software (F3). Wipe the pedestal dry.
   1 µL is often used for DNA blank and sample. A little more can make it more precise and accurate. TECAN NanoQuant requires 2 µL per well, best with all wells used individually blanked, not averaged.
4. **Measure:** Load 1–2 µL of your sample and click *Measure* (F1). Wipe dry. Repeat for remaining samples.
   Use the same volume as the blank.
5. **Post-Clean:** Wipe the pedestal again with the clean wet tissue and then with a *clean* dry portion, free of DNA.

Cell Culture

Microvolume: Do as above, but use the *Cell Culture* program with *Pedestal* option checked. Load no less than 2 µL of medium blank and cell culture. Make sure the culture is well-mixed before sampling.
To convert to the rough approximate equivalent 1 cm cuvette *E. coli* OD (at least for M9 media), multiply the microvolume OD600 by a factor of 7.5–9. When estimated to be in the desired OD range, measure the OD in a cuvette.

Cuvette: In the *Cell Culture* program, ensure the *Pedestal* option is unchecked. Blank with at least 500 µL medium, then at least 500 µL well-mixed sample culture. The cuvette can be gently shaken to further mix the culture, but avoid introducing bubbles.
An OD is only as reliable as the cell distribution uniformity of the source culture at the moment of sampling and the uniformity in the cuvette at the moment of measurement. Multiple readings may be averaged, with the cuvette shaken in between.

OD readings are only in a reliable window of linearity with cell concentration when OD600 = 0.1 – 1. When an OD is above this range or expected to be so, the culture must be diluted with the same medium before measuring, and the OD multiplied by the dilution factor. For example, 450 µL medium can be blanked, 50 µL culture mixed in to homogeneity, and then the OD reading multiplied by 10.

Note: The 2 mm sample beam passes through the cuvette 8.5 mm above the base of the cuvette.

Shyam's Sep 30, 2019 issue of *Lab Improvement Weekly*:

Some readers may be skeptical of using the Nanodrop microvolume pedestal for cell culture OD measurements.
I personally have always used cuvette measurements and thus require additionally culturing 2–3 × 700 µL extra volumes for measuring ODs in cuvettes in addition to what I need for an experiment that requires cells of a certain OD, *e.g.* mid-log phase. I found only 10–18% higher OD600 readings from the Nanodrop pedestal (2 µL) versus from cuvettes (700 µL) when the cultures were OD600=0.1–0.2 in M9CA. The conversion factor is pedestal OD × ~7.5–9 to get the equivalent cuvette measurement (including the 1 mm -> 1 cm pathlength conversion).
Cuvettes were loaded with pre-shaken cultures. Nanodrop samples were taken directly from measured cuvette samples, shaken to re-homogenize. 2 µL is necessary according to Nanodrop for reproducible microvolume measurements. Thus, Nanodrop measurements are a good primary estimator for OD600. But once your cultures are estimated to be in the proper OD range, you should still take a cuvette measurement if you need more accuracy, as when you need cultures diluted to an exact OD. So still plan 700 µL for that measurement. *Minimum Cuvette Volume*
Wonder how much liquid you need to make a minimal cuvette measurement? The manual says "Fill cuvettes with enough blanking or sample solution to cover instrument optical path (2 mm sample beam is 8.5 mm above cuvette bottom)."
I measure the bottom of the meniscus of 400 µL water in a standard cuvette to be 10 mm above the cuvette bottom. So long as there aren't bubbles below the surface, 400–500 µL should be a reproducible volume and allows you to cut the typically prescribe 1 mL measurements in half. I have switched my standard from 700 to 500 µL.

DNA concentration measurement precision between TECAN NanoQuant plate and Thermo NanoDrop, in ng/µL:

	Thermo NanoDrop	TECAN NanoQuant
pSPB1540	192.3	197.3
pSPB1541	174.3	178.4
pSPB1542	211.5	225.2
pSPB1543	240.3	242.7
pSPB1544 day 1	313.4	**325.7**
pSPB1545	255.2	267.4
pSPB1546	248.7	260.2
pSPB1547	277.1	284.4

pSPB1544 day2		**323.4**