

Protein Absorbance Measurements using the Thermo Scientific NanoDrop™ 2000/2000c Spectrophotometers

Q: Can I quantify Proteins using the NanoDrop 2000/2000c?

A: Yes. Options include the A280 method for purified proteins, a Proteins and Labels module for labeled antibodies and other protein incorporating fluorescent labels or intrinsic fluorescence. In addition colorimetric assays such as the BCA, Bradford, Modified Lowry and Pierce Protein 660 nm assay are easy and quick to run. Custom methods may also be set up via the Method Editor to analyze proteins, including peptides at 205 nm.

Proteins may be quantified using a micro-volume sample on the pedestal (2000 and 2000c) or using a standard cuvette (2000c).

Q: I am using a colorimetric method (e.g. Bradford, BCA etc..) to determine my protein concentration. Can I measure my sample using the A280 method on the NanoDrop instrument?

A: The A280 method is most applicable to purified proteins. Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry are generally used for uncharacterized protein solutions and cell lysates. If you are using a colorimetric assay now it is recommended that you continue to do so, and then run the sample (using the appropriate application) on the NanoDrop 2000/2000c to read absorption. If you are using a Bradford assay, you may find that the Pierce 660 nm assay is a better alternative (as described in Application Note from NanoDrop products).

Q: What are the sample size requirements for each instrument?

A: We recommend using a 2 uL sample size for pedestal-based protein measurements. Proteins and/or protein buffers may alter the surface tension properties of the solution and using the larger sample size is recommended to ensure proper column formation. As little as 0.5 uL can be used for precious high concentration samples.

Q: What is the dynamic (concentration) range for proteins that can be measured on the NanoDrop 2000/2000c spectrophotometers?

A: BSA concentrations between 0.1 mg/mL to 400 mg/mL can be measured using the A280 method. Refer to the user manual or our website for additional information regarding the detection limits for the Proteins and Labels module as well as for the various colorimetric assays.

Q: What sort of accuracy should I expect with the NanoDrop 2000/2000c?

A: Typically within 2%.

Q: What sort of reproducibility should I expect with the NanoDrop 2000/2000c?

A: e.g BSA in A280 module: +/- 0.1mg/mL for samples < 10 mg/mL and +/- 2% for samples > 10 mg/mL.

Q: Is simply wiping the pedestal surface enough to prevent carryover?

A: Yes. The highly polished quartz and stainless steel surfaces of the sample retention system are resistant to sample adherence, making the use of a dry laboratory wipe very effective in removing the sample.

Q: What additional analytes or biomolecules are routinely analyzed using the NanoDrop 2000/2000c?

A: These spectrophotometers are routinely used to determine the concentrations of dsDNA, RNA, ssDNA, fluorescent dyes incorporated into microarray and proteomic probes, microbial cell cultures and virtually any analyte that exhibits absorbance in the 190-840 nm range.

Q: How do I check the accuracy of the NanoDrop 2000/2000c?

A: CF-1 calibration check fluid should be used with the Calibration Check module of the instrument software. CF-1 is prepared from the NIST potassium dichromate standard SRM935 in acidified reagent grade water (Certificate of Analysis available from NanoDrop products).