Baseline Correction of Nucleic Acid & Protein A280 Measurements

Thermo Scientific NanoDrop Spectrophotometers use a bichromatic absorbance correction for nucleic acid and protein A280 measurements. This type of correction is performed to offset the effect of instrument noise and light scattering particulates on low concentration nucleic acid and protein sample measurements. Due to the lack of absorbance by nucleic acids or proteins at higher UV wavelengths, it has been our general observation that any UV wavelength at or above 320 nm can be utilized for this bichromatic correction.

Pedestal measurements made using NanoDrop™ spectrophotometers utilize shorter pathlengths than the classical 10 mm pathlength associated with most cuvettes, enabling measurements of highly concentrated samples. These concentrated samples have very high A260 nm or A280 nm values and therefore the normalization wavelength for the NanoDrop 1000, 2000/2000c and 8000 is positioned at 340 nm, an additional 20 nm further than the customary 320 nm. Similarly, the normalization wavelength for the NanoDrop Lite is positioned at 365 nm.

The software for the NanoDrop 1000 and NanoDrop 8000 Spectrophotometers automatically subtracts the 340 nm absorbance from the entire spectrum. The NanoDrop 2000/2000c software allows for the selection of any wavelength for this bichromatic correction (the default setting is 340 nm) or for de-selection of this function. The NanoDrop Lite subtracts the absorbance at 365 nm from the 260 nm and 280 nm wavelength absorbance, respectively.

NanoDrop spectrophotometers allow users to deselect the automatic bichromatic absorbance correction, in which case the absorbance at the correction wavelength can still be manually subtracted if desired.

For Technical Support, contact us at 877-724-7690, US & Canada, or worldwide at 302-479-7707 or send an email to nanodrop.techsupport@thermofisher.com.

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