Blank Measurements

Reference or "Blank" Measurement

When a Thermo Scientific NanoDrop Spectrophotometer is "blanked", a reference measurement of the transmitted light as a function of wavelengths is stored in memory. When a measurement of a sample is made, the intensity of light that has been transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance according to the following equation:

Absorbance = -log (Intensity$_{sample}$/Intensity$_{blank}$)

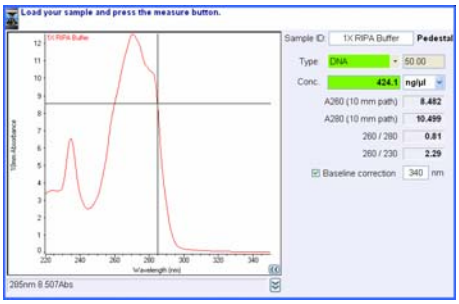
Thus, the measured light intensity of both the sample and of the blank are required to calculate the absorbance at a given wavelength. The Beer-Lambert equation is used to calculate the concentration from the absorbance:

A = e * b * c

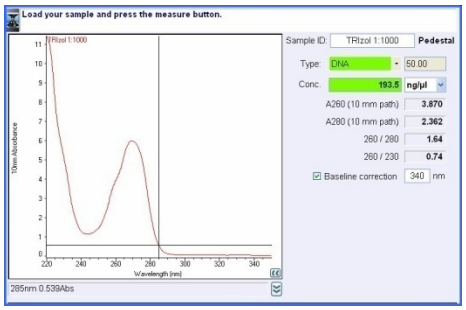
A is the absorbance value (in absorbance units), **e** is the wavelength-dependent molar absorbtivity coefficient (or extinction coefficient) with units of liter/mol-cm, **b** is the pathlength in centimeters, **c** is the analyte concentration in moles/liter or molarity (M).

Appropriate Blanking Solutions

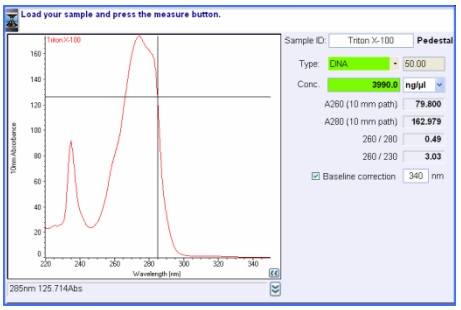
The blanking solution is generally the buffer at the same composition and pH as that of the unknown samples. There are, however, some buffers and reagents commonly used in life science laboratories which may not be suitable for absorbance measurements. RIPA buffers and detergents such as Triton X-100, as well as various nucleic acid isolation reagents such as Trizol, are examples of such reagents.



RIPA Buffer



Trizol



Triton X-100

Although the software displays a flat baseline when a buffer blank is measured, the spectrometer still detects the absorbance of the buffer solution when measuring an unknown sample. Very high absorbance values associated with particular components in a buffer may result in reduced signal being available for the unknown sample at the wavelengths of interest.

To assess the suitability of a blanking solution, blank a full spectrum Thermo Scientific NanoDrop spectrophotometer (e.g. a NanoDrop 2000 /2000c) using deionized water, and then measure the blanking solution as if it were a sample.

Verify that the buffer exhibits only minimal absorbance across the spectrum, especially at the analysis wavelength.

In the case of protein samples in RIPA buffers, one may use the BCA (bicinchoninic acid) Protein Assay rather than A280 nm measurements to determine a protein sample's concentration. A full spectrum instrument, such as the NanoDrop 2000/2000c or NanoDrop 8000, is ideally suited for this assay and pre-coded applications are included in the operating software. Check with the assay kit manufacturer to determine the RIPA buffer concentration compatible with the assay. Colorimetric assays such as the BCA Protein Assay are not compatible with the NanoDrop Lite. Use of a full spectrum instrument such as a NanoDrop 2000/2000c to make colorimetric measurements is required.

Storage or assay conditions may result in pH, ionic strength or individual component concentration changes to the starting buffer relative to the final buffer concentration of the unknown samples. Erroneous results will occur if the buffer used as the blank solution differs in composition from that of the sample buffer.

Reported negative sample concentration values are usually the result of making a blank measurement on a dirty pedestal. Always ensure that the pedestals are clean before making any blank or sample measurements. Negative sample values may also occur when a blank measurement is made using a solution that has more absorbance than the unknown sample has at the wavelength of interest.

In lieu of the spectral data available for blank assessment on the NanoDrop 1000, 2000/2000c, and 8000, the NanoDrop Lite spectrophotometer, requires a double blank measurement be performed prior to sample measurement. The second blank measurement confirms the quality of the initial blank before measuring samples.

Detection of low concentrations of samples may be problematic when using buffers that have high absorbance values at the measurement wavelength. The GenomiPhi DNA Amplification Kit is one example of a system where it is difficult to use absorbance spectroscopy to measure the concentration of the sample of interest. Quantification of unpurified amplification products will result in erroneous data due to the presence of kit components and sample proteins. In cases such as this, the use of a NanoDrop 3300 fluorospectrometer with a fluorescence assay (e.g. PicoGreen) may be more suitable.

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.