GENOVA NANO Technical Note: T09-004A

Direct UV protein measurement using the Genova Nano

Protein modes on the Genova Nano

The protein measurement mode allows the user to select a method from a list of common protein assays, including Pierce 660, BCA, Bradford, Lowry, Biuret and Direct UV. In the Direct UV mode, protein concentration can be measured directly at 280nm without using a calibration curve. However it is important to remember that there may be considerable error between different protein types as the determination is mainly a function of the tyrosine, tryptophan and phenylalanine content of a protein. Proteins with low aromatic amino acid content will have little or no absorbance at 280nm.

Direct UV requires readings at 280 and (optionally) 260nm and the result is calculated using the formula:

Protein concentration (µg/ml) = (A280 x 1550) – (A260 x 760)

This is based on the Warburg-Christian method and takes into account interference from nucleic acid contamination, often found in protein samples. Measurements can be made using the A280nm only if required. In this instance, the Factor 1 value should remain as set (1550), and the Factor 2 value should be set to 0.

Maximum and minimum detection limits

The maximum concentration of protein detectable using this method on a Genova Nano would be a concentration which would give an absorbance at 280nm of 2.5 at 0.2mm path length. Using the formula above and with a nominal value at 260nm of 90% the absorbance at 280nm (based on the results obtained from protein standards – see table below) the result would be as follows:

Protein concentration (µg/ml) = ((2.5x1550) – (2.25x760))x50 where 50 is the path length factor at 0.2mm.

So the maximum detectable concentration would be (3875-1710) x 50 = 108250µg/ml or 108mg/ml.

If the calculation is performed using a reading at 280nm only (i.e. Factor 2 is zero), the value would be about 194mg/ml.

The minimum detection limit can only be determined experimentally and will depend on the protein used. The data shown in Table 1 gives the absorbance values at 280nm and 260nm for a set of protein standards (Pre-Diluted Protein Assay Standards BSA set #23208, Thermo Scientific).

Standard (µg/ml)	A280nm	A260nm	Calculated concentration (µg/ml)
125	0.008	0.011	63
250	0.018	0.015	318
500	0.030	0.026	550
750	0.042	0.033	795
1000	0.055	0.041	1065
1500	0.073	0.051	1495
2000	0.122	0.116	2013

Table 1: Absorbance measurements for a set of seven protein standards together with the concentration calculated using the Warburg-Christian method used in the Direct UV mode. Measurements were taken at 0.5mm path length.

The lowest standards are just detectable above the background of the instrument but for more reproducible readings we would recommend a sample giving an absorbance of 0.05 or more. In this case this would indicate a protein concentration of at least 1mg/ml.