Genova Protocol: P09-006A

Direct UV determination of protein

Introduction

The direct UV method of protein determination has a number of advantages over traditional colorimetric assays in that it does not rely on an external protein standard and the sample is not consumed in the assay. However any non-protein component in the sample that absorbs in the UV region will interfere with the assay, as can insoluble or coloured components. In addition, different proteins will have different absorption coefficients leading to considerable error[1]. The most common use for the absorbance assay is to monitor fractions from chromatography columns, or whenever a quick estimation in protein concentration is required[2]. The direct UV assay is also recommended for calibrating bovine serum albumin or other pure protein solutions for use as standards in other methods. The direct UV method used on the Genova measures the sample at both 280nm and 260nm and includes a formula to correct for any nucleic acid interference.

Materials required

Quartz cuvette (e.g. Jenway 035 138)

Method

1. Turn on the Genova and allow to warm up.

2. From the main screen of the Genova select PROTEIN MODE and then DIRECT UV.

3. Select SETUP. Enter any sample dilution factor (sample volume + diluent volume) and adjust the units as appropriate. Select the required resolution.

4. Select EXIT and place a sample blank in the sample chamber. Close the lid. Press CAL and the instrument will calibrate at 280 and 260nm.

5. Remove the sample blank and insert the unknown sample. Close the lid. Press READ and the instrument will measure at 280 and 260nm.

6. Once the reading has been performed, the display will update to show the sample concentration and absorbance at both wavelengths.

Calculation

The concentration of the protein is calculated according to the following formula which is an approximation to the data of Warburg and Christian[1,3]:

Concentration (mg/ml) = (1.55 x A280) – (0.76 x A260)

References

1. Layne, E. Spectrophotometric and Turbidimetric Methods for Measuring Proteins. *Methods in Enzymology* **10***:* 447-455, (1957).
2. http://www.ruf.rice.edu/~bioslabs/methods/protein/abs280.html
3. Warburg, O. and Christian, W. *Biochem. Z.* **310**: 384 (1941).

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