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Technical Reference Guide

Determination of Protein Concentration

Introduction

Several methods are commonly used for determination of protein concentration. Measurement of the UV absorbance at 280 nm is most useful for pure protein solutions. Bradford and BCA assay methods are routinely used during protein purification and screening.

Protein Determination Using Absorbance at 280 nm

Determination of protein concentration by ultraviolet absorption (260 nm to 280 nm) depends on the presence of aromatic amino acids in proteins. Tyrosine and tryptophan absorb at approximately 280 nm. Higher orders of protein structure also may absorb UV light or modify the molar absorptivities of tyrosine and tryptophan, and thus the UV detection is highly sensitive to pH and ionic strength at which measurement is taken. Many other cellular components, and particularly nucleic acids, also absorb UV light. The ratio of A_{280}/A_{260} is often used as a criterion of the purity of protein or nucleic acid samples during their purification. The real advantages of this method of determining protein concentration are that the sample is not destroyed and that it is very rapid. Although different proteins will have different amino acid compositions and thus different molar absorptivities, this method can be very accurate when comparing different solutions of the same protein.

To make an accurate determination of protein concentration, you will have to produce a standard curve (A_{280}) with known amounts of purified protein. You will also have to provide a blank that is appropriate for the sample and contains the same concentrations of buffer and salts as the sample. It is often convenient to dialyze the sample and measure the absorbance of the sample (still in the dialysis sack) using the dialysis solution as the blank. Care must be taken to use quartz cuvettes, since glass absorbs UV light. A handy equation to estimate protein concentration that is often used is [Protein] (mg/mL) = $1.55*A_{280} - 0.76*A_{260}$

Protein Determination Using the Bradford Assay

The Bradford Assay, a colorimetric protein assay, is based on an absorbance shift in the dye Coomassie when bound to arginine and hydrophobic amino acid residues present in protein.

The (bound) form of the dye is blue and has an absorption spectrum maximum historically held to be at 595 nm. The anionic (unbound) forms are green and red. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the amount (concentration) of protein present in the sample.

Unlike other protein assays, the Bradford Protein Assay is less susceptible to interference by various chemicals that may be present in protein samples.

Protein Determination Using the BCA Assay:

This assay measures the formation of Cu^{+1} from Cu^{+2} by the Biuret complex in alkaline solutions of protein. The first reaction occurs at lower temperatures and is the result of the interaction of copper and BCA with the following residues: cysteine, cystine, tryptophan and tyrosine. At elevated temperatures, it has been shown that the peptide bond itself is responsible for color development. This is why performing the assay at 37°C or 60°C increases the sensitivity and reduces the variation in the response of the assay to protein composition. The BCA reagent forms a complex with Cu^{+}, which has a strong absorbance at 562 nm.

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