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Product Information

Total Protein Kit, Micro

Catalog Number **TP0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Protein determination is one of the most common operations performed in biochemical research. This Total Protein Kit can be used to determine the concentration of proteins in solution. The procedure is based on the procedure of Bradford,[1] who described in 1976 an *in vitro* micro-technique that employs the dye Brilliant Blue G.

When dissolved in an acid-alcoholic medium, the Brilliant Blue G dye reacts almost immediately with protein to form a blue-colored protein dye complex. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm. The amount of absorption at 595 nm is proportional to the protein present.[1]

This kit provides a simple, rapid, and highly sensitive method requiring only 50 µL or less of sample. Several publications have cited use of this kit in protein quantitation.[2-6]

Components

Protein Dye Reagent, 120 mL (Component Number B5809, not sold separately): Brilliant Blue G, 0.35 mg/mL, in phosphoric acid and methanol

Protein Standard Solution, 5 mL (Component Number P9744, not sold separately): Human albumin, 0.3 mg/mL, in saline with preservative

Equipment and Reagents Required But Not Provided

- Spectrophotometer capable of measuring absorbance at 595 nm
- Test tubes, 13 × 100 mm, disposable plastic
- Spectrophotometer cuvettes, polystyrene, disposable (e.g. Catalog Number C5416)
- Pipetting devices to deliver 0.05 mL and 2.5 mL
- 0.85% Sodium Chloride Solution (e.g. Catalog Number S0817)
  Note: This NaCl solution may be prepared by dissolving 8.5 g of sodium chloride in 1 L of water.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The Protein Assay Solution is prepared by mixing 1 volume of the Protein Dye Reagent (Component Number B5809) with 4 volumes of water in a plastic container.

Note: The Protein Assay Solution may appear turbid. This does not interfere with its performance in the procedure. However, if desired, the solution may be clarified by paper filtration.

The Protein Standard Solution is supplied ready for use.

Storage/Stability

Store this kit and its components at 2–8 °C.

The prepared Protein Assay Solution is stable for:

- Up to 2 weeks when stored at room temperature (18–26 °C)
- Or longer when stored refrigerated (2–8 °C)

Procedure

This procedure is linear to 0.5 mg/mL. Samples with concentrations greater than 0.5 mg/mL should be diluted two-fold with the 0.85% Sodium Chloride Solution and reassayed. The result for the diluted sample is then multiplied by 2.

The useful range of measurement of the procedure may be increased from 0.5 to 1 mg/mL by reducing the sample volume from 50 to 25 µL. The standard volume should also be decreased to 25 µL.

Certain drugs and other substances are known to interfere with protein determination.[1,7]

Due to the staining characteristics of the Protein Assay Solution, it is advisable to use disposable plastic cuvettes and pipettes. With continued use, glass cuvettes may develop a colored coating of dye, which may be removed by rinsing with methanol or ethanol.

1. Set up a series of labeled test tubes for Blank, Standard, and Tests.

2. Add 2.5 mL of the Protein Assay Solution to each test tube.

3. Add the following respective solutions to the labeled test tubes as follows:
   (a) To the test tube labeled Blank, add 50 µL of the Sodium Chloride Solution (Catalog Number S0817).
   (b) To the test tube labeled Standard, add 50 µL of the Protein Standard Solution (Catalog Number P9744).
   (c) To a test tube labeled Test, add 50 µL of sample.
   (d) Mix each tube thoroughly.

4. After approximately 2 minutes, transfer the solutions to separate cuvettes. Read and record the absorbance (A) of Standard ($A_{STANDARD}$) and Tests (A_{TEST}) versus the Blank as the reference at 595 nm. The color is stable for at least 30 minutes.

5. Calculate the protein concentration of the samples.

Calculation:

Protein concentration (mg/mL) =

$$\frac{(A_{TEST}) \times \text{Concentration of Standard}}{(A_{STANDARD})}$$

R**eferences**

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