3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Total Protein Reagent

Product Number **T 1949**
Store at Room Temperature

TECHNICAL BULLETIN

Synonym: Biuret Reagent

Product Description
Protein determination is one of the most common operations performed in biochemical research. The Total Protein Reagent can be used for the quantitative, colorimetric determination of total protein concentration in solution at 540 nm.

Various methods have been described to determine protein concentrations in biologic fluids based upon colorimetric, turbidimetric, electrophoretic, or immunologic procedures.[1,2] Colorimetric assays involving protein-dye binding or protein-reagent reactions are the most commonly used. The oldest and simplest of these assays is the biuret method. The biuret reaction involves addition of the biuret reagent, an alkaline copper salt solution, to the protein sample. A complex forms between the copper ions and opposing pairs of peptide bonded nitrogen atoms. Biuret reagent does not contain biuret, rather it will react with biuret.

Biuret reaction under alkaline pH conditions:

Copper ions + Proteins → Copper-Protein Complexes
(Purple)

The copper ions in the alkaline biuret reagent bind to the peptide bonds of proteins forming complexes with a purple color, which can be measured spectrophotometrically in the 540 nm region. The intensity of the color is proportional to the total protein concentration. The major drawback of the biuret assay is that the amount of protein required is in the milligram range. The described method for protein concentration determination with the Total Protein Reagent is based on a published procedure.[3]

The Total Protein Reagent is supplied as a ready-to-use liquid.

Component
The approximate composition of the product follows:

Sodium hydroxide	0.6 M
Copper sulfate	12.0 mM
Sodium, potassium tartrate	31.9 mM
Potassium iodide	30.1 mM

Reagents and Equipment Required But Not Provided
- Spectrophotometer capable of accurately measuring absorbance at 540 nm
- Cuvettes with optical properties for use at 540 nm
- Pipettes for the accurate delivery of volumes required
- Timer
- Appropriate Protein Standard

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the Total Protein Reagent at room temperature. The product is stable for at least 2 years. It is not suitable for use if a precipitate forms.

Procedure
All glassware must be free of protein films. Biological materials known to interfere with the biuret method include lipids, bilirubin, hemoglobin, and dextran.[4,5] Interference has also been observed with ammonium sulfate,[6-8] glycerol,[9] guanidine,[10] TRITON™ X-100,[10] TWEEN® 80,[10] and urea.[8]

The Total Protein Reagent is linear to 160 mg/ml. If the total protein level is greater than 160 mg/ml, dilute the sample with an equal volume of buffer and reassay. Multiply the result by 2 to compensate for the dilution.

1. Set up a series of test tubes labeled Reagent Blank, Standard, and Test Samples.
2. Pipette 1.0 ml of the Total Protein Reagent into each tube.
3. Add 20 µl of deionized water, protein standard, and test samples into the appropriately labeled test tubes. Mix by gentle inversion.
   Note: If cuvettes accommodate larger volumes, use 3 ml of the Total Protein Reagent and 60 µl of the sample.
4. Incubate each tube for 10 minutes at ambient temperature.
5. Read and record the absorbance at 540 nm (A_{540}) of all tubes versus the Reagent Blank as the reference. The biuret color is stable for at least 1 hour.
6. To determine total protein concentration (mg/ml) in the samples, refer to the Calculation section.

Calculation

The Total Protein Reagent is linear to 160 mg/ml. An absorbance change of 0.06 corresponds to a protein concentration of approximately 10 mg/ml with the described procedure.

Total Protein (mg/ml) =

$$\frac{A_{540}\text{ (sample)} \times \text{Concentration of Standard (mg/ml)}}{A_{540}\text{ (standard)}}$$

References

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