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ProductInformation

Total Protein Kit,
Micro Pyrogallol Red Method

Product Number **TP0400**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Various methods have been described to determine protein concentrations in biologic fluids, which are based upon colorimetric, turbidimetric, electrophoretic, or immunologic procedures.[1,2] The dye binding methods are characterized by good precision and sensitivity; however, they are subject to variations in binding to different proteins.[3,4,5,6,7] This micro pyrogallol red procedure is a modification of a published method.[7] The method is based upon measuring the shift in the absorption that occurs when the pyrogallol red-molybdate complex binds to basic amino acid groups of protein molecules. The increase in absorbance at 600 nm is directly proportional to protein concentration in the sample.

Components

Sufficient reagents are supplied for 240 assays. Both the Total Protein Reagent and the Protein Standard Solution are supplied ready-to-use.

Total Protein Reagent, micro pyrogallol red method (Product Code T 2074) – A buffered solution containing 0.05 mM pyrogallol red, 0.16 mM sodium molybdate with chelating agent, stabilizer, surfactant, and preservative.

2 x 120 ml

Protein Standard Solution, (Product Code P 8369)
A solution containing 0.5 mg/ml of human serum albumin in saline with 0.1% sodium azide as a preservative.

1 x 5 ml

Precautions and Disclaimer

This product is for laboratory research use only, not for drug, household, *in vitro* diagnostic, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Equipment and Reagents Required But Not Provided

- Spectrophotometer capable of measuring absorbance at 600 nm
- Test tubes or cuvets
- Pipettes for delivery of suggested volumes
- Timer
- Constant temperature water bath

Storage/Stability

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

Procedure

The Micro Pyrogallol Red Method is linear from 0.01–2 mg/ml. Samples with protein concentrations higher than 2 mg/ml should be diluted with an equal volume of isotonic saline and reassayed. Multiply the concentration obtained by 2 to compensate for the dilution.

The reactivity of the Total Protein Reagent for albumin and gamma globulins is equal. The relative reactivities of the Total Protein Reagent for kappa light chains and lambda light chains compared to human albumin are 1.57 and 0.85, respectively.

An absorbance change of 0.002 corresponds to a protein concentration of approximately 0.01 mg/ml, when a spectrophotometer is used for the measurement under the stated conditions.

Note: The total protein measurement is affected by less than 5% by the following:[8] inorganic phosphate, Ca^{2+} and Mg^{2+} ions, creatinine, urea, glucose, uric acid, and the sodium salts of citrate, oxalate, and ascorbate.

1. Set the spectrophotometer wavelength to 600 nm and the absorbance to zero using water as the reference.
2. Warm the Total Protein Reagent to room temperature, 30 °C, or 37 °C.
3. Set up a series of labeled test tubes for Blank, Standards, and Tests.
4. Pipette 1.0 ml of the Total Protein Reagent into each tube.
5. Pipette 20 µl (0.02 ml) of water, Protein Standard Solution, and samples to appropriately labeled test tubes. Mix by gentle inversion.
6. Incubate tubes for 3 minutes at assay temperature.
7. Read and record absorbance (A) of Blank, Standard, and Tests at 600 nm using water as the reference. Absorbance reading is stable for 15 minutes.
8. Subtract the absorbance of Blank from the absorbance of Tests and Standard to obtain change in absorbance (ΔA) due to protein.
9. Calculate the protein concentration of the sample.

Calculation:
Protein concentration (mg/ml) =

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

Example:

A_{BLANK} = 0.0676
A_{TEST} = 0.0829
$A_{STANDARD}$ = 0.1698
ΔA_{TEST} = 0.0829 – 0.0676 = 0.0153
$\Delta A_{STANDARD}$ = 0.1698 – 0.0676 = 0.1022

$$\text{Protein (mg/ml)} = \frac{0.0153}{0.1022} \times 0.5^{*} = 0.075$$

*Protein concentration (mg/ml) of standard

References

1. Grant, G.H., and Kachmer, J.F., The proteins in body fluid. in Fundamentals of Clinical Chemistry. Tietz, N.W., ed., WB Saunders (Philadelphia, PA: 1976), pp 358–374.
2. Cannon, D.C. et al., Proteins. in Clinical Chemistry - Principles and Technics, 2nd edition, Henry, R.J. et al., eds., Harper & Row (New York, NY: 1974), pp 422–431.
3. McElderry, L.A. et al., Six methods for urinary protein compared. Clin. Chem., **28**, 356–360 (1982).
4. Dilena, B.A. et al., Six methods for determining urinary protein compared. Clin. Chem., **29**, 553–557 (1983).
5. Pesce, M.A., and Strande, C.S., A new micromethod for determination of protein in cerebral spinal fluid and urine. Clin. Chem., **19**, 1265–1267 (1973).
6. Watanabe, N. et al., Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin. Chem., **32**, 1551–1554 (1986).
7. Fujita, Y. et al., Color reaction between pyrogallol red-molybdenum (VI) complex and protein. Bunseki Kagaku, **32**, E379–386 (1983).
8. Louderback, A. et al., A new dye-binding technique using bromocresol purple for determination of albumin in serum. Clin. Chem., **28**, 256-260 (1982).

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