TOTAL PROTEIN

(LIQUID)

4 x 60 ml

RE – ORDER TPR1310

INTENDED USE:

This reagent is intended for the quantitative in vitro determination of total protein in serum.

TEST SUMMARY:

Serum protein, by osmotic pressure, plays an important part in the maintenance of normal distribution of water between blood and tissue. The several fractions of serum protein vary independently and widely in disease. Low protein is caused by, among others, inadequate intake, impared synthesis, loss (as by hemorrhage), or excessive protein catabolism. High protein levels are caused mainly by dehydration.

The measurement of total serum protein accounts for a variety of different proteins whose concentrations may vary significantly and independently from each other, yet are reflected as a sum total (6).

The principal of this total protein assay is the biuret reaction. In alkaline solution, cupric ions react with all compounds with two amide or peptide bonds linked either directly or through an intermediate carbon atom (1) to form a violet colored complex. The intensity of this colored complex reflects direct proportionality to protein concentration over a fairly broad linear range (4).

REAGENT COMPOSITION:

Reactive ingredients:

Copper Sulfate	12 mmol/L
Potassium Iodide	30 mmol/L

Non-reactive ingredients:

Buffers, stabilizers and fillers

REAGENT PREPARATION:

The ClearChem Biuret Total Protein Reagent is in a final use form and no user-preparation is required.

REAGENT STORAGE AND STABILITY:

The reagent is stable in the unopened container until the expiration date on the label when stored at room temperature (18–24 °C).

PRECAUTIONS:

Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label. Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information.

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE:

Serum is the preferred specimen. Highly hemolyzed specimens should be avoided. Lipemic sera may interfere with test accuracy. Specimens are stable for at least one week at room temperature when held in tightly closed containers. Good practice would suggest refrigerated storage. Frozen samples should be thoroughly mixed upon thawing.

INTERFERING SUBSTANCES:

Young et al. (2) have published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including the determination of total protein.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 540 nm.

Matched cuvettes.

Constant temperature incubator set at 25 °C, 30 °C or 37 °C. Use the same temperature for assay of standard, controls and samples.

Distilled or deionized water.

Pipettes to measure water, reagent and samples.

TEST PROCEDURE:

Wavelength: 540 nm

Test: 2 mL reagent + 40 µL sample

Standard: 2 mL reagent + 40 µL of protein standard or known control

Blank: 2 mL reagent + 40 µL H_2O

Incubate: 25 °C, 30 °C or 37 °C (30 °C is recommended) for 15 minutes

Reading: Zero instrument with reagent blank. Read absorbance of samples and standards. The reaction end-point is stable for approximately 30 minutes.

CALIBRATION:

This assay requires the use of a protein standard. Use the standard provided with the reagent as directed or other commercially available standards or calibrators.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Commercially available control material with established values for total protein may be used.

CALCULATIONS:

The concentration of total protein in the sample is determined as follows:

$$\frac{\text{A sample}}{\text{A standard}} \times \text{conc. of std.} = \text{g/dL of total protein in sample.}$$

Sample Calculation:

If the absorbance of a specimen is 0.384 and the absorbance of 8 g/dL standard is 0.420:

$$\frac{0.384}{0.420} \times 8 = 7.3 \text{ g/dL of protein in sample.}$$

LIMITATIONS OF THE PROCEDURE:

Samples with total protein concentrations higher than 12 g/dL should be diluted with an equal volume of physiological saline (150 mmol/L sodium chloride in water) and assayed again; multiply the results by 2.

REAGENT PERFORMANCE:

Linearity: The assay is linear to 12 g/dL (3).

Correlation: Employing as a reference a commercial reagent based on an equivalent formulation (Gilford), results obtained with 72 serum samples of total protein concentration varying between 4.83 and 9.66 g/dL were compared with those obtained using the present reagent. The correlation coefficient was 0.975 and the regression equation was:

$y = 0.951x + 0.2818$.

Precision:

Within Run:			
Mean (g/dL)	4.84	5.67	6.97
SD	0.029	0.029	0.05
CV	0.599	0.511	0.717
N	10	10	10
Run-to-Run:			
Mean (g/dL)	4.86	5.69	7.10
SD	0.03	0.03	0.011
CV	0.617	0.527	0.155
N	30	30	30

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REFERENCE RANGE:

6.2 – 8.5 g/dl

1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than ambulatory. Differences may be as much as 1.2 g/dl.
2. It is strongly recommended that each laboratory establish its own Range.

REFERENCES:

White, A., Handler, P. and Smith, E.L., Principles of Bio-chemistry, 5th Ed., 111-112, McGraw-Hill Book Co., N.Y., 1973.

Young, D.S., Pestaner, L.C. and Gibberman, V., Clin. Chem. 21, 1975.

Faulkner, W.R. and Meites, S., Selected Methods of Clinical Chemistry, 9, 319, Amer. Assoc. for Clin. Chem., Washington, D.C., 1982.

Doumas, B., Standards for Total Serum Protein Assays, A Collaborative Study, Clin. Chem., 21, 1159 (1975).

Tietz, N.W., Clinical Guide to Laboratory Tests, 416, W.B. Saunders, Philadelphia, PA, 1983.

Kaplan, A. and Szabo, J., Clinical Chemistry: Interpretation and Techniques, 2nd Ed., 157, Lea & Febiger, Philadelphia, PA, 1983.

Manufactured For:
ClearChem Diagnostics