

Total Protein Reagent

INTENDED USE

For in vitro diagnostic use and for the quantitative determination of Total Protein in serum on the Olympus Demand® and Reply®.

SUMMARY

Proteins function as transport molecules and maintain normal blood volume by exerting colloidal osmotic pressure. The gamma globulins comprise an important part of the immune system. All the major plasma proteins except immunoglobulins are produced in the liver.¹

Total protein determinations are useful for detecting hyperproteinemia due to hemo-concentration and the various hyperglobulinemic conditions such as myeloma, infection, certain hepatic diseases and other pathological states associated with an increase in one or more of the protein fractions. Hypoproteinemia is observed in malnutrition, protein-losing kidney diseases, edema, hemorrhage and tissue wasting diseases.²

Methods for the determination of total protein have included a nitrogen determination (Kjeldahl), electrophoresis, refractive index, specific gravity measurement and colorimetric methods. Kingsley introduced the first simple procedure to be used in the clinical laboratory.³ Since then, the biuret method has become the method of choice for measuring proteins because of its simplicity, precision, and accuracy.

METHODOLOGY

Protein + Cu²⁺ → blue violet complex

The Thermo Total Protein Procedure is a modification of the biuret reaction as originally described by Kingsley.³ The blue violet chromogen is produced when cupric ions complex with the unshared electrons of the nitrogen and oxygen atoms of the protein peptide bonds. The intensity of the color produced is measured bichromatically at 540/660 nm, and is proportional to the amount of total protein present.

REAGENTS

Reactive Ingredients	Initial Conc.	Final Conc.
cupric sulfate	60 mmol/L	12.0 mmol/L
surfactant		

PRECAUTIONS

for in vitro diagnostic use. Do not ingest. Toxicity has not been established. Do not pipette by mouth. Avoid contact with skin, eyes and clothing. **The Packaging of This Product Contains Dry Natural Rubber.**

PREPARATION

Total Protein Reagent is ready to use as supplied.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date stated on the label when stored at 2°- 30°C.
2. After opening the reagent is stable for 28 days when stored on the Olympus Demand or Reply at 2°- 8°C.

DETERIORATION

1. The reagent should be a clear, blue solution. Turbidity or presence of black precipitate could indicate deterioration and the reagent should not be used.
2. Failure to achieve assay values on freshly prepared control sera could indicate deterioration.

SPECIMEN COLLECTION

1. Unhemolyzed serum is the recommended sample.⁴
2. Note the ambulatory or recumbent status of the patient. The normal total protein value of ambulatory patient serum is approximately 0.5 g/dL greater than recumbent patient.

SAMPLE STORAGE

Total Protein in serum is stable one week stored at room temperature or one month at 4°C.⁶

INTERFERING SUBSTANCES

1. At a Total Protein level of 5.1 g/dL, a positive interference was observed at a Triglyceride concentration of 330 mg/dL.
At a Total Protein level of 7.3 g/dL, a positive interference was observed at a Triglyceride concentration of 370 mg/dL.
2. No interference was observed at a Bilirubin concentration of 14 mg/dL.
3. At a Total Protein level of 7.3 g/dL, a negative interference was observed at a Hemoglobin concentration of 400 mg/dL.
4. Young has reviewed drug effects on serum creatinine levels.⁶

PROCEDURE

Test Parameters

Refer to the Thermo Reagent Applications for the Olympus Demand or Reply.

MATERIALS PROVIDED

Total Protein Reagent	10x18 mL
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MATERIALS REQUIRED BUT NOT PROVIDED

1. Olympus Demand or Reply system with Operator's Manual and Accessories.
2. Thermo Reagent Applications for the Olympus Demand or Reply.
3. Thermo Data-Cal (Cat. No. 1905-505 or TR43001) or equivalent.
4. Thermo Data-Trol N and Data-Trol A (Cat. No. 1902-050 or TR40001 and 1901-050 or TR41001) or equivalent.

STABILITY OF FINAL REACTION MIXTURE

The instrument automatically computes every determination at the same time interval.

CALIBRATION

Thermo Data-Cal (Cat No. 1905-505 or TR43001) or equivalent should be employed with each set of unknowns assayed.

LINEARITY

Linearity extends to 15 g/dL. Samples exceeding this value should be diluted with normal saline and repeated. Multiply the result by the dilution factor when calculating the unknown.

QUALITY CONTROL

Normal and abnormal control serum of known concentrations of Total Protein should be analyzed routinely with each group of unknown samples. Thermo's Data-Trol N and Data-Trol A (Cat. No. 1902-050 or TR40001 and 1901-050 or TR41001) are recommended for this purpose.

CALCULATION OF RESULTS

Results, expressed as g/dL at 37°C, are automatically calculated.

LIMITATIONS

The biuret reaction is not sufficiently sensitive to permit determination of total protein in fluids of low protein concentration such as urine and spinal fluid. It should be noted that in some cases a decrease in one fraction of the total protein tends to be offset by an increase in the other fractions. Therefore, the total protein determination may not reflect either the nature or the extent of an existing abnormality and may indeed even fail to indicate its presence. Determinations of albumin and/or globulin and calculation of the Albumin/Globulin (A/G) Ratio are suggested to aid in the differentiation.

$$\frac{\text{Albumin(g/dL)}}{\text{Protein(g/dL)} - \text{Albumin(g/dL)}} = \text{A/G Ratio Total}$$

$$\frac{\text{Total Protein (g/dL)} - \text{Globulin (g/dL)}}{\text{Globulin(g/dL)}} = \text{A/G Ratio}$$

EXPECTED VALUES

NORMAL RANGES:⁴ Ambulatory: 6.4 — 8.3 g/dL
Recumbent: 6.0 — 7.8 g/dL ' A/G Ratio: 1.1 —2.5

These ranges should serve only as guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist between laboratories and local populations.

PERFORMANCE CHARACTERISTICS

Precision

WITHIN-RUN	Level 1	Level 2
No. of Data Points	40	36
Mean g/dL	5.4	7.8
SD	0.038	0.049
CV%	0.7	0.6

TOTAL	Level 1	Level 2
No. of Data Points	40	36
Mean g/dL	5.4	7.8
SD	0.077	0.092
CV%	1.4	1.2

COMPARISON STUDIES

A comparison of the Thermo Total Protein reagent (y) with a commercial reagent of the same methodology (x) was performed on 40 human samples in a range of 4.8 - 9.2 g/dL. A correlation coefficient of 0.9916 was obtained; the linear regression equation was $y = 0.999x - 0.053$.

SENSITIVITY

Based on an instrument resolution of $A = 0.001$, this Thermo procedure has a sensitivity of 0.03 g/dL.

REFERENCES

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2. Hoffman, W.S., The Biochemistry of Clinical Medicine. 3rd ed., Year Book Medical Publishers, Chicago, 1966, p. 39.
3. Kingsley, G.R., Standard Methods of Clinical Chemistry, Academic Press, Inc., New York, 1972, Vol. 7, p. 199.
4. Tietz, N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1983, p. 416.
5. Tietz, N.W., Textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, 1986, p. 583.
6. Young, D.S., Effects of Drugs on Clinical, Laboratory Tests, AACC Press, Washington, D.C., 1990, p. 3-392—3-396.

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