TOTAL PROTEIN (BIURET) REAGENT SET

CAT NO : DT554 – 480

INTENDED USE
For the quantitative determination of total protein concentration in serum.

INTRODUCTION
Through osmotic pressure, serum protein is involved 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 primarily caused by malnutrition, impaired synthesis, loss (as by hemorrhage), or excessive protein catabolism. Elevated protein levels are caused mainly by dehydration.

The determination of total protein in serum makes use of the Biuret color reaction, known since 1878. Past attempts to stabilize the cupric ions in the alkaline reagent were unsuccessful until the addition of sodium potassium tartrate as a complexing agent. The present method for quantitative determination of total protein in serum is based on the method proposed by the American Association for Clinical Chemistry (AACC) and National Committee for Clinical Laboratory Standards (NCCLS).

PRINCIPLE
The enzymatic reaction sequence employed in the assay of Total Protein is as follows:

Alkaline Protein + Cu^{2+} \rightarrow Cu-Protein Complex

PH

Protein in serum forms a blue colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

REAGENT COMPOSITION
Total Protein Reagent contains the following:
- Sodium Hydroxide 600 mM
- Copper Sulfate 12 mM
- Sodium Potassium Tartrate 32 mM
- Potassium Iodide 30 mM
- Nonreactive ingredients.

Total Protein Standard: Bovine Albumin Ff. V with preservative 5.0 g/dl.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostics use. Caution: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The reagent contains sodium hydroxide which is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

STORAGE AND STABILITY
Store reagent at room temperature (18 – 25 °C). Store Protein standard refrigerated (2 – 8 °C).

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity or the presence of a black precipitate indicates reagent deterioration and should not be used. The reagent should be clear pale blue solution.

SPECIMEN COLLECTION
1. Test specimens should be serum and free from hemolysis.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera cause elevated results and should be run with a serum blank.
   a. Place 3.0 ml 0.9% saline in test tube.
   b. Add 0.05 ml (50 µl) sample.
   c. Zero spectrophotometer with 0.9% saline.
   d. Read and record absorbance of serum blank.
   e. Subtract blank absorbance from test absorbance.

4. Samples with bromsulfophthalein (BSP will result in falsely elevated results. Protein in serum is stable for one (1) week at room temperature (18 – 25 °C) and for at least one (1) month refrigerated (2 – 8 °C) when guraded against evaporation.

INTERFERING SUBSTANCES
Young et al has reviewed a number of drugs and substances that may affect protein concentration.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices (3 ml, 50µl)
2. Timing device.
3. Test tube and rack.
4. Spectrophotometer.

GENERAL INSTRUCTIONS
The reagent for Total Protein is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application available from us.

PROCEDURE (MANUAL)
1. Label tubes Blank, Standard, Controls, Patients, etc.
2. Pipette 3.0 ml of reagent into each tube.
3. Add 0.05 ml (50 µl) of standard and patients to appropriate tubes and mix by inversion.
4. Let the tubes stand at room temperature (18 – 25 °C) for ten (10) minutes.
5. Set spectrophotometer at 540nm and zero instrument with the reagent blank.
6. Read and record absorbance of each tube.

NOTE:
1. Final color is stable for sixty(60) minutes at room temperature.
2. Serums with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run, and the final answer multiplied by two (2).
3. ALTERNATE VOLUMES: 20 µl(0.02 ml) sample to 1.0 ml reagent. Calculations remain the same.

PROCEDURAL LIMITATIONS
The reagent is linear to 15.0 g/dl
1. Sample with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two (2).

2. The Biuret procedure is not sensitive at low ranges (<1 g/dl). Do not use for urine or spinal fluid.

**CALCULATIONS**

Abs. of Unknown

\[ \text{Abs. of Unknown} = \frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of Std} = \text{total protein (g/dl)} \]

Example:

Absorbance of unknown = 0.350
Absorbance of Standard = 0.400
Concentration of standard = 5 g/dl

\[ 0.350 \times 5 = 4.37 \text{ g/dl} \]

\[ 0.400 \]

**QUALITY CONTROL**

It is recommended that controls be included in each set of assays. Commercially available control material with established total protein values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration instrument malfunction or procedural errors.

**EXPECTED VALUES**

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 mandatory. Differences may be as much as 1.2 g/dl.

2. It is strongly recommended that each laboratory establish its own normal range.

**PERFORMANCE CHARACTERISTICS**

1. Linearity: 1.0 – 15.0 g/dl

2. Comparison: A comparison study when performed between this procedure and another procedure based on the same principle resulted in a correlation coefficient of 0.95 with a regression equation of \( y = 0.86x + 1.02 \)

3. Precision studies:

<table>
<thead>
<tr>
<th>Mean (g/dl)</th>
<th>S.D.</th>
<th>C.V.%</th>
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</thead>
<tbody>
<tr>
<td>6.8</td>
<td>0.12</td>
<td>1.8</td>
</tr>
<tr>
<td>3.7</td>
<td>0.08</td>
<td>2.1</td>
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**REFERENCES**


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