Total protein
Biuret Reagent

REF: 310 001 (2 x 100ml) 200 test
REF: 310 002 (4 x 100ml) 400 test
REF: 310 003 (8 x 100ml) 800 test
REF: 310 004 (2 x 500ml) 1000 test
REF: 310 005 (2 x 250ml) 500 test

Intended Use
Spectrum Diagnostics total protein reagent is intended for the in-vitro quantitative, diagnostic determination of total protein in human serum on both automated and manual systems.

Background
Plasma proteins are mainly synthesized in the liver and are involved in the maintenance of normal water distribution between tissues and blood, as well as acid-base balance. Due to some pathological conditions, both total protein level and the ratio of different fractions may change independently of one another. Hyperproteinemia may be detected during dehydration associated with diarrea or vomiting. The total protein levels also increase in multiple myeloma. Hyperproteinemia may occur as a result of prolonged low protein diet and in some pathological conditions such as nephrotic syndrome, bleeding, sprue, and salt retention.

Method
Colormetric method (Biuret reagent).

Assay Principle
In alkaline medium the copper reacts with the peptide bonds of proteins to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation, and potassium iodide prevents the auto-reduction of copper.

\[
\text{protein} + \text{Cu}^{2+} \xrightarrow{\text{Alkaline pH}} \text{Cu} – \text{protein complex}
\]

The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in the absorbance at 546nm.

Reagents
Standard Total protein (ST)
8.0 g/dL

Reagent (R)
- Sodium hydroxide: 750 mmol/L
- Copper sulfate: 12.0 mmol/L
- Sodium potassium tartrate: 40.9 mmol/L
- Potassium iodide: 19.8 mmol/L

(C)-Corrosive contains caustic materials.
K34 Causes burns.
S26-4S In case of contact with eyes, rinse immediately with plenty of water and seek medical advice in case of accident or if you feel unwell, seek medical advice immediately.

For further information, refer to the Total Protein reagent material safety data sheet.

Precautions and Warnings
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability
Spectrum Total protein reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles. The reagents are stable at 15 – 25°C. Only the standard is needed to be kept refrigerated at (2 - 8°C).

Deterioration
Do not use the total protein regents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

SYMBOLS IN PRODUCT LABELLING
- EC REA: Authorised Representative
- REF: Use by/Expiration Date
- LOT: For in-vitro diagnostic use
- REA: Batch Code/Lot number
- REF: CAUTION. Consult instructions for use
- LOT: Manufactured by
- Temp: Temperature Limitation

(C) - Corrosive

Specimen Collection and Preservation
Use serum or plasma (EDTA or heparin) for the test. Usually plasma results are higher due to fibrinogen. The serum or plasma should be separated from the cells within 4 hours.

Stability:
- 1 day at 15 – 25°C
- 4 weeks at 4 – 8°C
- 1 year at -20°C

System Parameters
- Wavelength: Hg 546 nm (530 – 570 nm)
- Optical path: 1 cm
- Assay type: End-point
- Direction: Increase
- Sample: Reagent Ratio
  - e.g.: Reagent volume: 1 ml
  - Sample volume: 20 μl
- Temperature: 15 – 25°C
- Zero adjustment: Reagent blank
- Incubation time: 10 minutes at 15 – 25°C
- Sensitivity: 1.0 g/dL
- Linearity: 12 g/dL

Procedure

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (R)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>20 μl</td>
<td>-----</td>
</tr>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Mix. Incubate for 10 minutes at room temp. Measure absorbance of specimen (A specimen) and standard (A standard) against reagent blank within 30 minutes.

Calculation

\[
\text{Serum protein conc. (g/dL)} = \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times 6
\]

Note: For turbid highly icteric sera, prepare a serum blank by adding 20 μl serum to 1 ml saline into a labeled test tube. Read absorbance of serum blank at 540 nm vs water and subtract serum blank absorbance from test absorbance before calculating results.

Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Mean (g/dL)</td>
<td>5.2</td>
</tr>
<tr>
<td>SD</td>
<td>0.12</td>
</tr>
<tr>
<td>CV%</td>
<td>2.47</td>
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</table>

Run to run (Repeatability)

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Mean (g/dL)</td>
<td>5.7</td>
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<tr>
<td>SD</td>
<td>0.19</td>
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<tr>
<td>CV%</td>
<td>2.53</td>
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Methods Comparison

A comparison between Spectrum Diagnostics Total Protein reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.978 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 1.0 g/dL.

Linearity

The reaction is linear up to total protein concentration of 12 g/dL. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances

Serum, plasma

Hemolysis

No interference up to hemoglobin level of 7.5 g/L.

Icterus

No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia

No significant interference.

Drugs

Sera from patients receiving dextran may cause artificially high levels due to turbidity during color development. This positive bias can be minimized by centrifuging the reaction mixture before reading the absorbance.

Expected Values

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<tbody>
<tr>
<td>Adults</td>
<td>6.6 – 8.7 g/dL</td>
</tr>
<tr>
<td>Children</td>
<td>(&gt; 1 year)</td>
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<tr>
<td></td>
<td>6.0 – 8.0 g/dL</td>
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<tr>
<td></td>
<td>(&lt; 1 year)</td>
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<tr>
<td></td>
<td>4.8 – 7.6 g/dL</td>
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<tr>
<td>Newborns</td>
<td>(&lt; 4 weeks)</td>
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<tr>
<td></td>
<td>4.6 – 6.8 g/dL</td>
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<tr>
<td>Prematures</td>
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<td>3.4 – 5.0 g/dL</td>
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Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

1.0 – 12 g/dL.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal. 
SS6: dispose of this material and its container at hazardous or special waste collection point. 
SS7: use appropriate container to avoid environmental contamination. 
SS1: avoid release in environment. Refer special instructions/safety data sheets.

References


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<tr>
<th>ORDERING INFORMATION</th>
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<tbody>
<tr>
<td>CATALOG NO.</td>
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<td>310 004</td>
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<td>310 005</td>
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