Total protein FS*

Diagnostic reagent for quantitative in vitro determination of total protein in serum or plasma on photometric systems

Order Information
Cat. No. Kit size
1 2311 99 10 021 R1 4 x 20 mL + R2 1 x 20 mL
1 2311 99 10 026 R1 5 x 80 mL + R2 1 x 100 mL
1 2311 99 10 023 R1 1 x 800 mL + R2 1 x 200 mL
1 2311 99 10 704 R1 8 x 50 mL + R2 8 x 12.5 mL
1 2311 99 90 314 R1 10 x 20 mL + R2 2 x 30 mL
1 2300 99 10 030 + R2 6 x 3 mL Standard
1 2311 99 10 026 R1 5 x 80 mL + R2 1 x 200 mL
1 2311 99 10 023 R1 1 x 800 mL + R2 1 x 200 mL
1 2311 99 90 314 R1 8 x 50 mL + R2 8 x 12.5 mL
1 2300 99 10 030 + R2 6 x 3 mL Standard

Summary [1,2]
Measurement of total protein is a useful test in a variety of disorders. Decreased total protein concentrations can be detected in defective protein synthesis in the liver, protein loss due to impaired kidney function, intestinal malabsorption or nutritional deficiency. Elevated protein levels occur in chronic inflammatory disorders, liver cirrhosis and dehydration.

Method
Photometric test according to biuret method

Principle
Proteins form a violet blue color complex with copper ions in alkaline solution. The absorbance of the color is directly proportional to the concentration.

Reagents
Components and Concentrations
R1: Sodium hydroxide 100 mmol/L
Potassium sodium tartrate 17 mmol/L
R2: Sodium hydroxide 500 mmol/L
Potassium sodium tartrate 80 mmol/L
Potassium iodide 75 mmol/L
Copper sulphate 30 mmol/L
Standard: 5 g/dL

Storage Instructions and Reagent Stability
The reagents and the standard are stable up to the end of the indicated month of expiry, if stored at 2 – 25°C and contamination is avoided. Do not freeze the reagents and protect them from light!

Warnings and Precautions
1. Reagent 1: Warning. H290 May be corrosive to metals. P234 Keep only in original container. P390 Absorb spillage to prevent material damage.
3. The reagents contain sodium hydroxide. Do not swallow! If the reagents get in contact with skin or mucous membranes rinse immediately with water!
4. Total Protein Standard FS contains animal material. The standard should be handled as potentially infectious and with the same precautions used for patient specimens.
5. In serum or plasma of patients who have received large intravenous amounts of polydextans, too high values can be measured with the biuret method. In such cases an alternative method (e.g. Kjeldahl) has to be used.
6. In very rare cases, samples of patients with gammopathy might give falsified results [5].
7. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
8. For professional use only!

Waste Management
Please refer to local legal requirements.

Reagent Preparation
The standard is ready to use.

Sample Start
Mix 4 parts of R1 with 1 part of R2 (e.g. 20 mL R1 + 5 mL R2) = mono reagent
Stability after mixing: 1 year at 2 – 25 °C

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Specimen
Serum or plasma
Stability [3]: 6 days at 20 – 25°C
4 weeks at 4 – 8°C
at least one year at –20°C

Freeze only once!
Discard contaminated specimens!

Assay Procedure
Application sheets for automated systems are available on request.

Wavelength 540 nm, Hg 546 nm
Optical path 1 cm
Temperature 20 – 25°C/37°C
Measurement Against reagent blank

Substrate start

<table>
<thead>
<tr>
<th>Sample or standard</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. water</td>
<td>20 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Mix, read absorbance A1 after 1 – 5 min. at 20 – 25 °C/37 °C, then add:</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 min. at 20 – 25°C/37°C and read absorbance A2 within 60 min.

\[ \Delta A = (A2 - A1) \] sample or standard
**Sample start**

<table>
<thead>
<tr>
<th>Sample or standard</th>
<th>Blank</th>
<th>Sample or standard</th>
<th>20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. water</td>
<td>20 µL</td>
<td>Dist. water</td>
<td>-</td>
</tr>
<tr>
<td>Mono reagent</td>
<td>1000 µL</td>
<td>Mono reagent</td>
<td>1000 µL</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 min. at 20 – 25°C/37°C and read absorbance against the reagent blank within 60 min.

\[ \Delta A = \frac{A_{Sample/Standard}}{A_{Blank}} \]

**Calculation**

With standard or calibrator,

\[
\text{Total protein [g/dL]} = \frac{\Delta A \times \text{Conc. Std / Cal.}}{\text{Sample} \times \text{Calibrator} / \text{Cal.}}
\]

**Calibrators and Controls**

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned values of the calibrator are traceable to the biuret method. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 063 20 X 3 mL</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062 20 X 5 mL</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062 20 X 5 mL</td>
</tr>
</tbody>
</table>

**Performance Characteristics**

**Measuring range**

The test has been developed to determine total protein concentrations within a measuring range from 0.05 – 15 g/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

**Specificity/Interferences**

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL, dextran up to 2000 mg/dL and lipemia up to 1000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [4].

**Sensitivity/Limit of Detection**

The lower limit of detection is 0.05 g/dL.

**Precision (at 37°C)**

\[
\begin{array}{ccc}
\text{Intra-assay} & \text{Mean [g/dL]} & \text{SD [g/dL]} & \text{CV [%]} \\
\text{n = 20} & & & \\
\text{Sample 1} & 5.27 & 0.05 & 0.91 \\
\text{Sample 2} & 7.05 & 0.07 & 1.01 \\
\text{Sample 3} & 10.4 & 0.08 & 0.80 \\
\text{Inter-assay} & \text{Mean [g/dL]} & \text{SD [g/dL]} & \text{CV [%]} \\
\text{n = 20} & & & \\
\text{Sample 1} & 5.24 & 0.06 & 1.06 \\
\text{Sample 2} & 7.07 & 0.11 & 1.53 \\
\text{Sample 3} & 10.4 & 0.14 & 1.32 \\
\end{array}
\]

**Method Comparison**

A comparison of DiaSys Total protein FS (y) with a commercially available test (x) using 68 samples gave following results:

\[ y = 1.00 \times 0.07 \text{ g/dL}; r = 0.997 \]

**Reference Range**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>[g/dL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>6.6 – 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - 30 day(s)</td>
<td>4.2 – 6.2</td>
<td>4.1 – 6.3</td>
</tr>
<tr>
<td>1 – 6 month(s)</td>
<td>4.4 – 6.6</td>
<td>4.7 – 6.7</td>
</tr>
<tr>
<td>6 months – 1 year</td>
<td>5.6 – 7.9</td>
<td>5.5 – 7.0</td>
</tr>
<tr>
<td>1 – 18 year(s)</td>
<td>5.7 – 8.0</td>
<td>5.7 – 8.0</td>
</tr>
</tbody>
</table>

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

**Literature**


**Manufacturer**

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

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DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany