**TOTAL PROTEIN**

**BIURET METHOD**

**Principle:**
Colorimetric determination of the total protein based on the principle of Biuret reaction (copper salts in an alkaline medium). Protein in plasma or serum forms a blue coloured complex when treated with cupric ionic in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

**Reagent Concentration:**
- Biuret Reagent:
  - Sodium iodide: 100 mmol/l
  - Potassium sodium tartrate: 15 mmol/l
  - Copper sulphate: 5 mmol/l
  - Potassium Iodide: 15 mmol/l
- Standard:
  - Albumin Bovine Fraktion V: 7 g/dl

**Preparation and Stability**
Reagent and Standard are ready to use. The reagents are stable up to the stated expiry date when stored at +2 to +8 ºC.

**Samples:**
Serum, heparinised or EDTA-plasma.
Stability in serum at +2 to +8 ºC is up to 1 month and at +15 to +25ºC is up to 1 week.

**Procedure:**
- Wavelength: Hg 540nm (530-570nm)
- Temperature: +25ºC
- Cuvette: 1cm light path
- Zero adjustment: Reagent blank
  - Each series needs one reagent blank only

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent/R1</td>
<td>1000µl</td>
<td>1000µl</td>
</tr>
<tr>
<td>Standard/R4</td>
<td>---</td>
<td>25µl</td>
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<tr>
<td>Serum or Plasma</td>
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</table>

Mix. Incubate for 15 minutes at 30-37C. Wait for 5 minutes at room temperature. Measure absorbance of sample and standard against reagent blank.

**Calculation:**

**By Standard:**
- ?A sample
- \( \frac{\text{A sample}}{\text{standard}} \times \text{conc (g/dl)} = \text{Protein in g/dl} \)
- ? A standard
- Standard concentration: 7g/dl

**Linearity:**
The method is linear up to 15g/dl or 150g/l. If the protein concentration is greater than 15 g/dl in the serum or plasma, dilute sample 1:2 with saline solution and repeat test. Multiply result by 2.

**Normal Values:**

| Serum, Adults: | 6.7-8.7 g/dl (67-87 g/l) |
| Newborn: | 5.2-9.1 g/dl (52-91 g/l) |
| Children: | 5.4-8.7 g/dl (54-87 g/l) |

**Notes:**
The sample blank for clear, colourless sera is equivalent to 0.2 g/dl and is therefore negligible. A sample blank must be determined for haemolytic and lipemic sera by pipetting 20µl serum to 1000µl physiological saline and measurement against distilled water. The absorbance of the sample blank has to be subtracted from the absorbance of the sample.

**Quality Control:**
For accuracy and reproducibility control:-
- Assayed Multi-Sera Normal and Elevated.
For reproducibility control:-
- Multi-Sera Low, Normal and Elevated.

**Presentation:**
- TOP0350 2 x 125ml, 250 tests
- Biuret Reagent 2x125ml
- Standard 1x5ml

**Safety precautions:**
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Health and Safety data sheets are available on request.

**Literature:**