



TOTAL PROTEIN

Biuret Method

Cat.No. 101-0240

Size 3 x 100 ml

Cat.No. 101-0033

Size 4 x 250 ml

PRINCIPLE:

Colorimetric determination of total protein is based on the principle of the Biuret reaction.

The peptide bonds of protein bind with copper ions (Cu^{2+}) in alkaline solution to form a blue-violet complex. Tartrate is added as a stabilizer while potassium iodide is used to prevent auto-reduction of the alkaline copper complex. The intensity of the blue-violet complex is proportional to the protein concentration.

SAMPLE:

Serum, heparinized or EDTA-plasma

Stability up to 1 month at +2 °C to +8 °C or 1 week at +15°C to +25°C.

REAGENTS:

| | |
|---------------------------|------------|
| 1. Biuret reagent | |
| Potassium sodium tartrate | 32 mmol/L |
| Potassium iodide | 30 mmol/L |
| Copper sulphate | 18 mmol/L |
| Sodium hydroxide | 200 mmol/L |

| | |
|-------------|--|
| 2. Standard | |
| Albumin | Standard concentration see on the vial label |

All the reagents are ready for use.

Reagents are stable up to the expiry date when stored at +2 °C to +8°C.

PROCEDURE:

| | |
|------------------|------------------|
| Wavelength: | 540 nm |
| Cuvette: | 1 cm light path |
| Temperature: | +20 °C to +25 °C |
| Color stability: | 30 min |
| Zero: | reagent blank |

| Pipette into test tubes | Reagent blank | Standard | Sample |
|-------------------------|---------------|----------|---------|
| Biuret reagent | 1000 µl | 1000 µl | 1000 µl |
| Standard | - | 20 µl | - |
| Sample | - | - | 20 µl |

Mix and let stand at +20 °C to +25 °C for 10 minutes.
Measure the absorbance of the sample and standard against the reagent blank.

CALCULATION:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{stand.conc.} = \text{g/L protein}$$

EXPECTED VALUES:

| | |
|-----------------------|-------------|
| Normal born babies | 46 - 70 g/L |
| Children from 3 years | 66 - 87 g/L |
| Adults | 66 - 87 g/L |

LINEARITY:

up to 120 g/L

QUALITY CONTROL:

| | | |
|----------|-----------|-------------------|
| CONTRO-N | 20 x 5 ml | Cat. No. 101-0083 |
| CONTRO-P | 20 x 5 ml | Cat. No. 101-0084 |

NOTE:

1. A sample blank must be determined for hemolytic and lipemic sera by pipetting 20 µl sample to 1000 µl physiological saline and measurement against distilled water. The absorbance of the sample blank has to be subtracted from the absorbance of the sample.
2. The sample blank for clear, colorless sera is equivalent to 2 g/L and is therefore negligible.
3. Biuret reagent contains sodium hydroxide which is caustic. In the case of contact with skin and mucous membranes, flush affected area with large quantities of water and seek immediate medical attention.
4. The standard contains sodium azide as preservative. Avoid ingestion or contact with skin or mucous membranes.

REFERENCES:

1. Weichselbaum, T.E. Amer.J. Clin. Path., 1946, 16, 40-48
2. Josephson, B. and Gyllensard, C., Scand. J. Clin. Lab. Invest. 1957, 9, 29