



## Total protein

Biuret. Colorimetric  
For In-Vitro and professional use only  
Store at 2° to 8° C

### PRINCIPLE OF THE METHOD

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant.  
The intensity of the color formed is proportional to the total protein concentration in the sample<sup>a</sup>.

### CLINICAL SIGNIFICANCE

The proteins are macromolecular organic compounds, widely distributed in the organism. They act like structural and transport elements. The proteins of the serum are divided in two fractions, albumin and globulins  
The determination of total proteins is useful in the detection of:  
- High protein levels caused by hemoconcentration like in the dehydrations or increase in the concentration of specific proteins.  
- Low protein level caused by hemodilution by an impaired synthesis or loss (as by hemorrhage) or excessive protein catabolism<sup>a</sup>.  
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

### REAGENTS

|                      |  |            |
|----------------------|--|------------|
| <b>R</b><br>Biuret   | Sodium potassium tartrate              | 15 mmol/L  |
|                      | Sodium iodide                          | 100 mmol/L |
|                      | Potassium iodide                       | 5 mmol/L   |
|                      | Copper (II) sulphate                   | 19 mmol/L  |
| <b>T PROTEIN STD</b> | Bovine albumin primary standard 7 g/dL |            |

### PRECAUTIONS

Copper (II) sulphate: Environmentally dangerous (N): R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.

### PREPARATION

The reagents are ready to use.

### STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

### Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm  $\geq$  0.22.

### ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 540 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

### SAMPLES

Serum or heparinized plasma

Stability of the sample: 1 month at refrigerator (2-8°C).

### PROCEDURE

- Assay conditions: Wavelength: 540 (530-550) nm  
Cuvette: ..... 1 cm. light path  
Temperature ..... 37°C / 15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

|               | Blank | Standard | Sample |
|---------------|-------|----------|--------|
| R (mL)        | 1.0   | 1.0      | 1.0    |
| Standard (µL) | --    | 25       | --     |
| Sample (µL)   | --    | --       | 25     |

- Mix and incubate 5 min at 37°C or 10 min at room temperature.
- Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

### CALCULATIONS

A Sample

$\times 7$  (Standard conc.) = g/dL of total protein in the sample

A Standard

### QUALITY CONTROL

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

### REFERENCE VALUES<sup>1</sup>

Adults: 6.6 – 8.3 g/dL

Newborn: 5.2 – 9.1 g/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

### PERFORMANCE CHARACTERISTICS

**Measuring range:** From detection limit of 0,20 g/dL to linearity limit of 15 g/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

### Precision:

| Mean (g/dL) | Intra-assay (n=20) |      | Inter-assay (n=20) |      |
|-------------|--------------------|------|--------------------|------|
|             | 5.07               | 9.64 | 5.15               | 9.74 |
| SD          | 0.04               | 0.08 | 0.06               | 0.14 |
| CV (%)      | 0.88               | 0.90 | 1.23               | 1.43 |

**Sensitivity:** 1 g/dL = 0,07 A.

**Accuracy:** Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.9918.

Regression equation:  $y = 1.0164x - 0.1264$ .

The results of the performance characteristics depend on the analyzer used.

### INTERFERENCES

Hemoglobin and lipemia<sup>a</sup>.

A list of drugs and other interfering substances with total protein determination has been reported by Young et. al

### NOTES

- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.

### BIBLIOGRAPHY

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