TOTAL PROTEIN

Biuret Method

Intended Use:
Total protein Test reagent/kit is a medical device intended for estimation of Total protein in serum or plasma.

Clinical Significance
Proteins are constituents of muscle, enzymes, hormones and several other key functional and structural entities in the body. They are involved in the maintenance of the normal distribution of water between blood and the tissues. Consisting mainly of albumin and globulin the fractions vary independently and widely of diseases. Increased levels are found in dehydration. Decreased levels are found in malnutrition, impaired synthesis, protein losses as in hemorrhage or excessive protein catabolism.

Principle
Protein+ Cu^{++}Blue ⟶ Violet coloured complex
The intensity of the colour formed is directly proportional to the amount of total proteins present in the sample.

Reagent Composition

R1 Biuret Reagent	Copper Sulphate- 20 mmol/L Sodium Potassium tartarate – 15mmol/L Sodium Hydroxide – 30 mmol/L
StdConc	6 g/dl

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Store at R1 Biuret Reagent at Room Temp or below 30ºC.Store standard at 2-8ºC
All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.Store the vials tightly closed protected from light and preventcontaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 555 nm (520 – 570 nm).
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Proteins are reported to be stable in serum or plasma for 6 days at 2-8ºC.

Assay Procedure
Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	**Blank**	**Std**	**Test**
R1 Biuret Reagent	1000µl	1000µl	1000µl
Distilled water	10 µl	-	-
Protein Standard (S)		10 µl	
Sample		-	10 µl

Mix well and incubate at 37^{0}C for 5 mins. or at R.T (<30°C) for 10 mins. Measure the absorbance of the Standard (Abs. S) and Test Sample (Abs.T) against the Blank, at 555 nm (520-570) within 60mins.

Calculations

$$\text{Total Protein in g/dl} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 6$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity
The procedure is linear up to 15 g/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Reference Values
Serum, Plasma : 6.0 – 8.0 g/dl
It is recommended that each laboratory establish its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	555 nm (520-570)
Blank with	Reagent
Sample Volume	10 µl
Reagent Volume	1000 µl
Std Conc.	6 g/dl
Incub. Temp.	R.T. (30°C)
Incub. Time	5 min.
Delay Time	5 sec
Normal Range	6 g/dl To 8 g/dl
Linearity	Up to 15 g/dl
Unit	g/dl

Notes
1. Gross haemolysis, ampicillin and heparin interfere with the results. Elevated bilirubin and lipemic samples may have a slight effect on accuracy. For grossly lipemic samples run a sample blank by adding 0.02 ml sample in 2 ml distilled water. Read the absorbance against D.W. and substract the blank absorbance.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. Gornall, A.G., et al, (1949) Biol. Chem. 177:751
2. Doumas, B.T. (1975) Clin Chem. 21:1159

For *in vitro* Diagnostic use only.