# *ANA BIO CLR* TOTAL PROTEIN

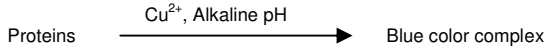
(Biuret Method)

**Intended Use**

Total protein is a reagent kit used for the determination of total protein in serum or plasma based on Biuret method. The reagents are for *in vitro* diagnostic use.

**Principle**

Proteins react with cupric ions under alkaline pH to produce a coloured complex. The coloured complex absorbs light at 546 nm (530 - 570 nm). The intensity of the colour is directly proportional to the protein concentration in specimen.




**Reagent Provided**

1. Biuret Reagent - Ready to use.
2. Standard - Total Protein (6 gm/dl).

**Reagent storage and stability**

Biuret reagent and standard are stable till the expiry date indicated on the bottle label when stored at 2° - 8℃.

**Specimen collection and preservation**

Blood should be collected in a clean dry container. Plastic or siliconized container should be avoided as it may prolong clotting time. Serum or plasma should be separated from the cells within 60 minutes. For plasma separation following anticoagulants may be used.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- HEPARIN 200 IU/ml of blood
- OXALATE 3 mg/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Proteins are stable in the serum and plasma for 7 days when stored at 2° - 8℃ and for a month when stored at -20℃ or frozen.

**Assay guidelines for Analyzers**

| | |
|---|---|
| Reaction type | End point with standard |
| Reaction slope | Increasing |
| Incubation time | 5 minutes at 37°C |
| Wavelength | 546 nm (530 - 570 nm) |
| Blank | Reagent Blank |
| Blank absorbance limit | < 0.300 Abs. against distilled water blank. |
| Sample volume | 20 µl (0.02 ml) |
| Reagent volume | 1000 µl (1.0 ml) |
| Standard concentration | 6 gm/dl |
| Factor calculation | 6 gm/dl ÷ Abs. of Std. |
| Low Normal | 6.3 gm/dl |
| High Normal | 8.4 gm/dl |
| Linearity | Up to 18 gm/dl |

**Assay guidelines for Manual procedure**

Bring the reagent and standard to room temperature before performing the assay.

| **Reagents** | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| Biuret Reagent | 1000 µl (1.0ml) | 1000 µl (1.0ml) | 1000 µl (1.0ml) |
| Standard | - | 20 µl (0.02 ml) | - |
| Sample | - | - | 20 µl (0.02 ml) |

1. Mix thoroughly and incubate at 37°C for 5 minutes.
2. Read the absorbance against reagent blank at 546 nm (530 - 570 nm).
3. The final colour is stable for 1 hour, if not exposed to light.

**Calculation**

$$\text{Con. in sample (gm/dl)} = \frac{\text{Sample OD}}{\text{Std OD}} \times \text{Con. of Std.}$$

**Normal Range**

Guidance value : 6.3 - 8.4 gm/dl

**Note**: Expected range varies from population to population and each laboratory should establish its own normal range.

**Limitation**

- The working reagent is considered unsatisfactory and should not be used if it develops insoluble precipitate.
- The reagent is linear up to 18 gm / dl. For higher value, dilute sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
- For haemolysed or icteric samples (Bilirubin > 5 mg %) a saline blank should be run along with the assay. Read the absorbance of saline blank against distilled water and subtract it from sample absorbance.

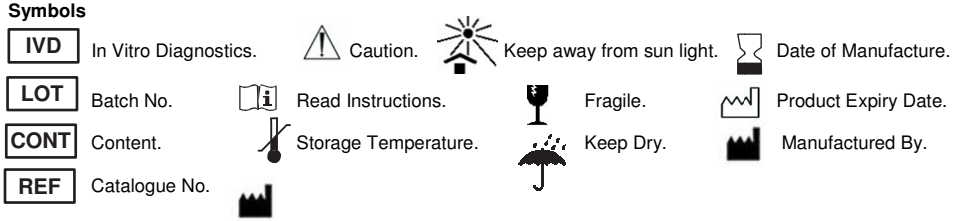
**Quality Control**

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

**References**


1. Stirkland R.D., et al. Anal, Chem. 33, (1961).
2. Henry, R.J., et al, "Clinical Chemistry- principles and technics" Harper & Row, II ed. (1974).


**Symbols**





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