# TOTAL PROTEIN

## (Biuret) End-Point

BIO LAB DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016
CE

## CLINICAL SIGNIFICANCE

Decreased levels are associated with malnutrition and hypoalbumlnaemia. Increased levels are observed in dehydration and hyper-globulinaemia.

## PRINCIPLE

In the presence of alkaline cupric sulphate, the proteins produce a **VIOLET** colour. Intensity of the colour is proportional to protein concentration.

## REAGENTS COMPOSITION

| | |
|---|---|
| **1. Biuret Reagent** | **Q.S.** |
| Cupric sulphate | 6 mMols/L |
| Sodium and Potassium tartarate | 21 mMols/L |
| Sodium Hydroxide | 750 mMols/L |
| **2. Standard (Protein)** | **Q.S.** |
| Value of Protein Standard | 8.0 Gms/dL |

### Working Reagent Preparation

All reagents are ready to use.

## STORAGE AND STABILITY

**Biuret (Reagent No. 1)**

Stable at 18-25°C till expiry stated on label.

**Protein Standard**

Stable at 2-8°C till expiry stated on label.

***Do not freeze the Standard.***

## SAMPLE

Serum or plasma which has no sign of haemolysis. Common anticoagulants have no interference on this assay. ***Avoid haemolysed serum or plasma.***

## SYSTEM PARAMETERS

| | |
|---|---|
| Reaction | End-Point |
| Temperature | 37°C |
| Wavelength | 550 ± 20 nM |
| Absorbance Range | 0-2 Å |
| Cuvette Path Length | 1 cM |
| Reaction Time 37°C | 5 Minutes |
| Protein Standard Value | 8.0 Gms/dL |
| Linearity | 15.0 Gms/dL |
| Max. limit of blank rgt. | 0.4 Å |
| Final Colour Stability | 30 Minutes |

| | |
|---|---|
| **Reagent Volume** | **500 µL** |
| **Sample Volume** | **20 µL** |

## PROCEDURE FOR AUTO ANALYSERS

| | |
|---|---|
| Reagent 1 ..... | 500 µL |
| Sample or Standard ..... | 20 µL |

Mix well. Incubate at 37°C for 5 minutes or at RT for 10 minutes. Read at 550 nM (± 20 nM) against blank. The final colour is stable for apx. 30 mins.

**NOTE** : Progrmame the analyser using system parameters. A specific programme data sheet may be provided for each analyser upon request.

## MANUAL PROCEDURE

| **1. Pipette into 3 Test Tube .....** | **Blank** | **Standard** | **Test** |
|---|---|---|---|
| Reagent No. 1 ..... mL | 1.0 | 1.0 | 1.0 |
| Standard ..... µL | - | 0.05 | - |
| Sample ..... µL | - | - | 0.05 |

2. Mix well. Incubate for 5 minutes at 37°C or 10 minutes at RT.
3. Read at 550 nM (550 ± 20 nM) or **GREEN** filter against Blank.
4. The final colour is stable for approximately 30 minutes.

## RESULTS

**Compute**

$$\text{Total Proteins in Gms/dL} = \frac{\Delta \text{ O.D. Test}}{\Delta \text{ O.D. STD}} \times 8$$

## EXPECTED VALUES

6.5 to 7.5 Gms/dL Proteins.

As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations.

## LIMITATIONS

Colour development gradually increases on prolongation of time more than 30 minutes specified in the procedure.

***This assay is linear up to 15 Gms/dL proteins.***

For values higher than 15 Gms/dL repeat test with serum diluted in 0.9% sodium chloride solution. Multiply result by dilution factor applied i.e. multiply by 2 for a 1:1 dilution.

## NOTE

**This method is not useful for Microprotein / C.S.F. Protein assay.**

**(For Microprotein assay, BIOLAB offers C.S.F. Protein Kit - Turbidometry method).**

## WARNING

This reagent system is for ***invitro*** use only.

This reagent system contains preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incidents wash off with plenty of water or consult a physician.

## QUALITY CONTROL

To ensure adequate quality control, each kit should be tested against a standard control sera. It should be realised that the use of quality control material checks both instrument and reagent function together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glasswares and accuracy of pipetting. It is appropriate to establish each laboratory's accuracy constant and interpret values accordingly.

## BIBLIOGRAPHY


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N. TIETZ, Fundamentals of Clin. Chem., W.B. Saunders Company, Philadelphia P.A.



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