

TOTAL PROTEIN

BIURET Method



DIATEK

CLINICAL SIGNIFICANCE :

The assay kit is for determination of serum total proteins (TP). 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 in diseases. Increased levels are found mainly in dehydration. Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in hemorrhage or excessive protein catabolism.

TEST PRINCIPLE :

Total proteins bind with the Cu^{++} in a buffered medium to form a coloured complex. The intensity of the colour formed is directly proportional to the amount of total proteins present in the sample at 546nm.

REAGENTS COMPOSITION :

R1 (Biuret Reagent) :

Potassium iodide : 30.7 mmol/L,
Potassium sodium tartrate: 42.5 mmol/L
Copper sulfate: 12 mmol/L,
Sodium hydroxide: 300 mmol/L,
Surfactant and Stabilisers

Standard :

Protein Standard : 4 gm/dL

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 °C, and contaminations prevented during their use. Do not use reagents over the expiration date. The reagent is ready-to-use. Once opened the reagent is stable for 1 month On-board the fully-auto analyser at approximately 10°C. Once opened the reagent is stable upto 1 month at 2-8°C.

KIT CONTENTS

Pack size :	CODE No. TP01 (2x50 ml)	CODE No. TP02 (5 x 100 ml)
Reagent 1 Biuret Reagent	2x50 ml	5 x 100 ml
Protein Standard (4 gm/dL)	1x1 ml	1x2 ml

SAMPLES : Serum or heparinised Plasma

Serum should be separated from blood as soon as possible.

MATERIALS REQUIRED BUT NOT PROVIDED :

Total Protein Control (Use of assayed QC sera is recommended to validate test result).

INTERFERENCES :

There was no interference observed in test result with Hemoglobin upto 500 mg/dl, Intralipid upto 1000 mg/dl, Bilirubin upto 40 mg/dl and Ascorbic Acid upto 30 mg/dl

ASSAY CONDITIONS:

Wavelength :	546 nm (540 - 555)
Cuvette:	1 cm light path
Constant temperature	37°C
Reaction	End Point
Standard Conc.....	4.0
Linearity.....	13 gm/dl
Unit.....	gm/dl
Slope of Reaction.....	Increasing
Blanking.....	Reagent

PROCEDURE :

Pipette into test tubes labeled Blank (B), Standard (S) and Test (T) as follows:

	B	S	T
Biuret Reagent	1.0 ml	1.0 ml	1.0 ml
Protein Standard		20 µl	
Specimen			20 µl

Mix and incubate for 5 mint at 37°C. Read absorbance of Standard (S) and Test (T) against Blank (B) with 546 nm. The final color is stable for 1 hour at R.T.

CALCULATIONS :

$$\text{Total Protein in gm/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 4$$

REFERENCE RANGE :

6.0 to 8.3 gm/dl

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Use clean glassware and microtips while pipetting Total Protein Standard. Replug Total Protein Standard vial after use
3. Avoid contamination of the reagent during the assay process.
4. Before the assay begins, bring all the reagents to room temperature.
5. If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen/standard.
6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
7. Programmes for specific autoanalysers are available on request.
8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to Protein concentration of 13 gm/dl. The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 2 with Normal Saline and multiply the result by 2.

BIBLIOGRAPHY :

1. Gornall A. et al., Determination of serum proteins by means of the biuret reaction, J. Biol. Chem., 177, (1949), 751.
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4. Vassault, A. Grafmeyer, D. Naudin, et al., Protocole de validation de techniques. (Document B, stade 3) Ann. Biol. Clin., (1986), 44, 686.