

# TOTAL PROTEIN (BIURET Method-End Point)

## Intended Use

The reagents are used for the quantitative determination of Total Protein in serum or plasma. For in-vitro diagnostic use only.

## Introduction

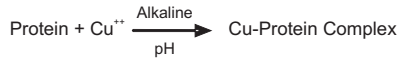
A total serum protein test measures the amount of two major groups of proteins in the blood: albumin and globulin. Plasma proteins derive primarily from synthesis in the liver, lymph nodes, spleen and bone marrow. In disease states both the total plasma protein level and the ratio of the individual fractions may be dramatically altered from their normal values. Hypoproteinemia may be caused by malnutrition, extensive bleeding, sprue (deficient protein absorption), kwashiorkor (acute protein starvation) etc. and hyperproteinemia may be observed in severe dehydration and disease states such as multiple myeloma.

## Method

Biuret method- End Point.

## Principle: End point analysis

Protein in serum forms a blue purple colored complex when reacted with cupric ions in alkaline solution.



Intensity of color is directly proportional to the concentration of total protein in serum or plasma, when measured at 546nm.

## Reagent Composition

### Reagent 1:

Sodium Hydroxide	100 mmol/l
K-Na Tartrate	16 mmol/l
Copper Sulphate	6 mmol/l

### Reagent 2:

Total protein standard	6 gm/dl
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## Precautions

Following precautions should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

## Reagent Storage and Stability

Unopened Reagent 1 is stable till expiry as mentioned on the label when stored at 15-30°C.

Standard Reagent 2 is stable till expiry as mentioned on the label when stored at 2-8°C. Close the Standard vial soon after use to avoid deterioration.

**Note:** On request, Reagent 3 ( Total protein: 10 g/dl) & Reagent 4 (Total protein: 12 g/dl) can be provided for linearity check with Reagent 2 (Total protein: 6 g/dl-Standard) .

## Reagent Preparation

Ready for use.

## Reagent Deterioration

Reagents should be clear. Turbidity and/or precipitation may be because of deterioration.

## Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 3 days at 2-8°C or 1 month at -20°C .

## General Assay Parameters

Mode	End Point
Wavelength 1 (nm)	546
Wavelength 2 (nm)	-
Blank with	Reagent
Sample Volume (µl)	10/20
Reagent R1 (µl)	500/1000
Incubation Time (min)	15 / 5
Incubation Temperature(°C)	RT/37
Normal Low (g/dl)	6
Normal High (g/dl)	8.7
Linearity (g/dl)	Upto 12
Conc. of Standard (g/dl)	6
Units	g/dl

## Procedure

One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

	Blank	Standard	Sample
Reagent 1	1000µl	1000µl	1000µl
Dist. Water	20µl	-	-
Reagent 2	-	20µl	-
Sample	-	-	20µl

Mix well & incubate for 15 min at room temperature or 5 min. at 37°C. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 546nm.

## Calculation

Total Protein concentration in the sample can be calculated using the following formula:

$$\text{Total Protein (g/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (g/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.180} \times 6 = 6.6 \text{ g/dl}$$

If the total protein concentration exceeds 12g/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

## Reference value

### In serum:

Infants	4.8 – 7.6 g/dl
Adult	6.6 - 8.7 g/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

## Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample:reagent ratio remains same.
2. Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample blank may be used as mentioned below:
  - Add 20µl of serum sample to 1000µl of normal saline (instead of distilled water) and read absorbance at 546nm.
  - Subtract the absorbance obtained as above, from the absorbance of Sample. Use this corrected absorbance for calculation.

## Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for total protein.

## Performance

**Linearity Limit:** 12g/dl

## Precision:

## Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (g/dl)	5.35	6.96
S.D.	0.04	0.05
C.V. %	0.64	0.66

## Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (g/dl)	5.35	6.96
S.D.	0.03	0.05
C.V. %	0.57	0.66

## References

1. Fschbach FT, Dunning MB III, eds.(2004). Manual of Laboratory and Diagnostic Tests, 7th ed. Philadelphia: Lippincott Williams and Wilkins. Kennedy JW, Carey RN, Coolen RB, et al.
2. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (Ep5- A). Wayne, PA: The National Committee for Clinical Laboratory Standards, 1999. [www.qualtextlabs.org/pdf/Total-Protein-Insert-0406.pdf](http://www.qualtextlabs.org/pdf/Total-Protein-Insert-0406.pdf)
3. Tietz NW. Clinical guide to laboratory test, 2nd ed. Saunders Co., 1991.
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd. Ed., AACCC Press, Washington DC, 1990, 3-104 thru 3-106.

## Pack Presentation

Product Code/ Catalogue no.	Pack Size*	Reagent 1	Reagent 2
KGTPR101.2.1	2x50ml	2x50ml	1x2ml
KGTPR101.2.2	5x50ml	5x50ml	1x3ml

\* Pack size may vary on market demand

Revision No: (Ver: KGTPR101.2/1)

Date of Issue: (1st April, 2010)

## Symbols

Following symbols are used in the labeling of KEE GAD kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions
	In Vitro Diagnostics		Storage temperature
	Expiry Date		Content
	Product Name		Manufactured By



**Manufactured by:**  
**KEE GAD Biogen Pvt. Ltd.**  
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