

# TOTAL PROTEIN – LR

(Biuret Method)



## INTENDED FOR USE:

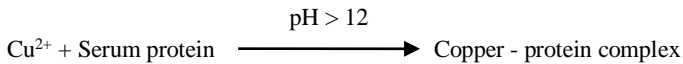
The Total protein - LR is an *in vitro* assay for the quantitative determination of Total protein in serum, plasma, Urine and CSF.

## CLINICAL SIGNIFICANCE:

Total protein estimation is useful for monitoring gross changes in protein levels caused by various disease states. The quantitative analysis of total protein alone is of limited diagnostic value. But quantitative determinations of total protein in conjunction with other tests such as serum albumin, liver function tests (or) protein electrophoresis, calculation of albumin globulin ratio is useful in the diagnosis of various diseases. Total protein levels are increased in the multi conditions of dehydration, diarrhea, multiple myeloma and macroglobulinemia. Total protein levels are decreased in malnutrition and starvation.

## PRINCIPLE:

Copper ions react in alkaline solution, with protein peptide bonds to give a purple coloured biuret complex. The amount of complex formed is directly proportional to the amount of protein in the specimen.



## REAGENT COMPOSITIONS:

### R1 Biuret Reagent

Sodium Hydroxide	200 mmol/L
Potassium Iodide	30 mmol/L
Copper Sulphate	18 mmol/L
Sodium Potassium Tartrate	30 mmol/L
Surfactant	qs
Preservative	qs

### R2 Protein Standard

Bovine Serum Albumin	5.5 gm/dL
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## REAGENT STORAGE:

Biuret reagent is stable at room temperature (15-30°C) till the expiry and Protein standard is stable at 2-8°C until the expiry date mentioned on the label. Reagents are ready to use and should be protected from direct light

## SAMPLE COLLECTION:

Unhemolyzed fresh serum, plasma with heparin or EDTA. Albumin is reported to be stable in the sample for 6 days at 2-8°C.

## PRECAUTIONS & WARNING:

Avoid pipette with mouth. The preparation, according to current regulation, is classified as not dangerous. The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation. Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

## SYSTEM PARAMETERS:

Reaction Type	:	End Point Method
Wave Length	:	555 nm
Optical path length	:	1 cm
Abs max	:	≤ 0.2
Flow cell Temperature	:	37°C
Blank	:	Reagent
Incubation Time	:	5 min
Reagent Volume	:	1000 uL
Sample Volume	:	10 uL
Standard Concentration	:	5.5
Reaction Slope	:	Increasing
Low Normal	:	6.0
High Normal	:	8.3
Linearity	:	10.0
Units	:	gm/dL

**Note:** Gross haemolysis, ampicillin and heparin interfere with the results. Elevated bilirubin and lipaemic samples may have a slight effect on accuracy. For grossly lipaemic samples run a sample blank by adding 0.02 mL sample in 2 mL distilled water. Read the absorbance against distilled water and subtract the blank absorbance from the test absorbance. The reagent may be used in semi and fully automated analyzers.

## ASSAY PROCEDURE:

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

Addition Sequence	Blank (µL)	Standard (µL)	Test (µL)
R1 Biuret Reagent	1000	1000	1000
R2 Protein Standard	...	10	...
Sample	...	...	10

Mix well and incubate for 5 minutes at 37°C. Read absorbance of the Standard (S) and Test (T) against the Blank (B) at 555 nm (520 – 570).

**CALCULATION:**

$$\text{Total Protein in gm/dL} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 5.5$$

**SYMBOLS USED ON THE LABELS:****SENSITIVITY / LIMIT OF DETECTION:**

The method will accurately measure Total Protein level is 2.0 gm/dL.

**LINEARITY:**

The procedure is linearity up to 10 gm/dL. If values exceed this limit dilute the sample with normal saline and repeat the assay. Calculate the value using the proper dilution factor.

**QUALITY CONTROL:**

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent function.

**REFERENCE RANGE:**

Adults : 6.4 – 8.3 gm/dL

Children : 6.0 – 8.0 gm/dL

**Note:** It is recommended that each laboratory establish its own normal range representing its patient population.

**INTERFERENCE:**

No interference was observed by up to hemoglobin 0.3 mg/dL, glucose 500 mg/dL, Triglycerides 300 mg/dL, bilirubin 30 mg/dL.

**PRECISION:**

Precision studies were performed with two controls using NCCLS protocol EP5 –A. The results of the precision studies are shown below:

Sample	Within - Run		Between - Run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control - 1	6.51	1.25	6.72	1.85	13.23	3.10
Control - 2	4.38	1.05	4.45	1.25	8.83	2.30

**REFERENCES:**

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