LIQUIZYME

TOTAL PROTEIN

(Biuret Method)

BEACON

Code	Product Name	Pack Size
LS038D	Liquizyme Total Protein	1 x 120 ml
LS038E	Liquizyme Total Protein	5 x 120 ml
LS038F	Liquizyme Total Protein	10 x 120 ml

Intended Use

Diagnostic reagent for quantitative in vitro determination of Total Protein in human serum and plasma.

Clinical Significance

Total protein is useful for monitoring gross changes in protein levels caused by various disease states. It is usually performed in conjunction with other tests such as serum albumin, liver function tests or protein electrophoresis. An albumin/globulin ratio is often calculated to obtain additional information.

Increased levels of serum protein are observed in dehydration, multiple myeloma and chronic liver disease.

Decreased levels are encountered in renal diseases and terminal liver failure.

Principle

Biuret method. The peptide bonds of protein react with copper II ions in alka line solution to form a blue-violet ion complex, (the so called biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tartrate is added as a stabiliser whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520-560).

Reagent Composition

Reagent 1: Biuret Reagent

Copper II Sulphate	:	<10 mmol/l
Potassium Sodium Tartrate	:	>20 mmol/l
Potassium Iodide	:	>0.6 mol/l
Sodium Hydroxide	:	742 mol/l

Reagent 2 : Total Protein Standard : 6 gm/dl

Ready to use

Reagent Preparation

Reagents are liquid, ready to use.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at +2–+8°C.

Material Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

Specimen Collection And Handling

Use unheamolytic serum or plasma (heparin, EDTA)

It is recommended to follow NCCLS procedures (or similar standardized conditions).

Stability

6 days	:	at +20 – +25°C
4 weeks	:	at +4 – +8°C

Discard contaminated specimens.

Calibration

Calibration with the Total Protein standard provided in the kit is recommended.

Quality Control

Its recommended to run normal and abnormal control sera to validate reagents performance.

Unit Conversion

gm/dl x 10 = gm/L

Expected Values

Serum : 6.0 to 8.0 gm/dl

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification	:	0.37 gm/dl
Linearity	:	15 gm/dl
Measuring range	:	0.37 – 15 gm/dl

Precision

Intra-assay precision Within run (n=20)	Mean (gm/dl)	SD (gm/dl)	CV (%)
Sample 1	5.84	0.05	0.94
Sample 2	4.82	0.10	2.03

Inter-assay precision Run to run (n=20)	Mean (gm/dl)	SD (gm/dl)	CV (%)
Sample 1	2.62	0.05	1.83

Comparison

A comparison between Total Protein (y) and a commercially available test (x) using 20 samples gave following results :

y = 1.003 x - 0.005 gm/dl

r = 0.999

Interferences
Following substances do not interfere:
haemoglobin up to 7.5 gm/l, bilirubin up to 40 mg/dl, triglycerides up to 1500 mg/dl.

Warning And Precautions
For in vitro diagnostic use. To be handled by entitled and professionally educated person.
R1 contains 3.0 % sodium hydroxide.

Waste Management
Please refer to local legal requirements.

Assay Procedure
Wavelength : 546 nm
Cuvette : 1 cm

Addition Sequence	Reagent Blank	Standard	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Distilled Water	10 µl	-	-

Mix and incubate for 5 minutes at room temperature. Measure the absorbance of the standard absorbance and sample absorbance against the reagent blank, within 60 minutes.

Calculation

$$\text{Total protein (gm/dl)} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 6$$

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	End point
Wavelength 1 (nm)	546
Sample Volume (µl)	10
Reagent Volume (µl)	1000
Incubation time (min.)	5
Incubation temp. (°C)	Room Temperature
Normal Low (gm/dl)	6
Normal High (gm/dl)	8
Linearity Low (gm/dl)	0.37
Linearity High (gm/dl)	15
Standard Concentration	6 gm/dl
Blank with	Reagent
Unit	gm/dl

References

1. Cornall, A. G., Bardawill, C. J., David, M. M.: J. Biol. Chem. 177, 751, 1949.
2. Doumas, B. T., Bayse, D. D. a kol.: Clin. Chem. 27, 1642, 1981.
3. Chromý, V., Fischer, J.: Clin. Chem. 23, 754, 1977.
4. Chromý, V., Fischer, J., Vozníèek, J.: Z. Med. Labor.-Diagn. 21, 333, 1980.
5. Tietz Textbook of Clinical Chemistry and Molecular diagnostics. Burtis, C.A.,
6. Ashwood, E.R., Bruns, D.E.; 5th edition, WB Saunders Company, 2012.

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

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