BIOLABO
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TOTAL PROTEIN Biuret Method
Ready-to-use Liquid

Reagent for quantitative determination of total protein in human serum and plasma.

REF LP87016 **R1** 1 x 200 mL **R2** 1 x 5 mL

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CE

IN VITRO DIAGNOSTIC

CLINICAL SIGNIFICANCE (1)

The overall composition of a patient's plasma or serum should be studied first by determining its total protein content and then its composition by electrophoresis.
Decrease in the volume of plasma water (hemoconcentration), noted in dehydratation (severe vomiting, diarrhea, Addison's disease, or diabetic acidosis), is reflected as relative hyperproteinemia. Hemodilution (increase in plasma water volume) occurring with water intoxication or salt retention syndromes, during massive intravenous infusions, and physiologically when a recombant position is assumed, is reflected as relative hypoproteinemia. Hypoproteinemia due to low levels of albumin in plasma is also common and has many causes. Mild hyperproteinemia may be caused by an increased in the concentration of specific proteins (infection). Marked hyperproteinemia may be caused by high levels of monoclonal immunoglobulins produced in multiple myelomia and other malignant paraproteinemias.

PRINCIPLE (4) (5)

Colorimetric method described by Gornall and al. The peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a coloured complex which absorbance, proportional to the concentration of total protein in the specimen, is measured at 550 nm. The Biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution.

REAGENTS

R1	**TOTAL PROTEIN**	Reagent
Sodium hydroxide	370	mmol/L
Na-K Tartrate	10	mmo/L
Potassium iodide	3	mmol/L
Copper II sulfate	3	mmol/L

Danger:

Met Corr.1: H290 - May be corrosive to metals
Eye Dam.1: H318 - Causes serious eye damage
Skin Corr. 1B: H314- Causes severe skin burns and eye damage
P234: Keep only in original container, P264: Wash hands thoroughly after handling, P280 : Wear protective gloves/protective clothing/eye protection/face protection, P301+330+331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting, P303+361+353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water, P304+P340 : IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing, P305+351+338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing, P310: Immediately call a POISON CENTER or doctor/physician. Classification due to: Sodium Hydroxide 2,5-< 10%
For more details, refer to Safety data sheet (MSDS)

R2 **TOTAL PROTEIN** Standard

Bovine Albumin 6 g/dL

According to 1272/2008 regulation, this reagent is not classified as dangerous

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Analyzer

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).
- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

REAGENTS PREPARATION

Ready for use

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 18-25°C, and used as described, reagents are stable:

Unopened:
- Store standard (vial R2) at 2-8°C
- Until expiry date stated on the label of the kit

Once opened:

Transfer the requested quantity and well cap the original vial.
- Reagent (vial R1) is stable at least 1 year at 18-25°C
- Store standard (vial R2) at 2-8°C

Discard reagent if cloudy or if absorbance at 550 nm > 0.150.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or plasma. Analyse fresh or store at 2-8°C less than 72 h.

Total protein in serum is stable for:
✓6 months at –20°C
✓indefinitely at -70°C.

LIMITS (3)

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

CALIBRATION (6)

- REF 95015 BIOLABO Multicalibrator traceable to SRM 927
- Standard (vial R2)

The calibration frequency depends on proper instrument functions and on the preservation of reagent

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with | H2O Demineralized water | Biological hazard

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level 1
- REF 95011 BIOLABO EXATROL-P Level 2
- External quality control program

It is recommended to control in the following cases

- At least once a run
- At least once within 24 hours
- When changing vial of reagent
- After maintenance operations on the instrument

If control is out of range, apply following actions:

1. Repeat the test with the same contro.
2. If control is still out of range, prepare a fresh control serum and repeat the test
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test
4. If control is still out of range, calibrate with a new vial of reagent
5. If control is still out of range, please contact BIOLABO technical support or your local Agent

EXPECTED VALUES (2)

In serum or plasma

Total Protein	**(g/dL)**
In cord	4.8-8.0
Premature	3.6-6.0
Newborn	4.6-7.0
1 week	4.4-7.6
7 days-1 year	5.1-7.3
1 year-2 years	5.6-7.5
≥ 3 years	6.0-8.0
Adult, ambulatory	6.4-8.3
Adult, recombent	6.0-7.8
≥ 60 years	Lower by 0.2

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES at 37°C on KENZA 240TX

Linearity Range: between 0.7 and 8.0 g/dL

Detection limit: approx. 0.01 g/dL

Precision:

Within-run N = 20	***Low level***	***Medium level***	***High level***
Mean (g/dL)	3,53	6,89	9,14
S.D. g/dL	0,03	0,07	0,08
C.V. %	0,85	0,96	0,89

Between run N = 20	***Low level***	***Medium level***	***High level***
Mean (g/dL)	3,54	6,87	9,06
S.D. g/dL	0,06	0,10	0,15
C.V. %	1,68	1,41	1,61

Comparison studies with commercially available liquid reagent:

Realised on human specimens (n=116) between 2.7 and 8.8 g/dL

y = 0.9652 x + 0.2395 r = 0.9895

Analytical sensitivity: approx**.** 0.057 abs (1 g/dL)

Interferences:

Turbidity	Positive interference from 0.114 abs
Total bilirubin	No interference up to 541 µmol/L
Direct bilirubin	No interference up to 397 µmol/L
Glucose	No interference up to 1059 g/dL
Ascorbic acid	No interference up to 2500 g/dL
Haemoglobin	Positive interference from 128 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 2 months

Calibration Stability: 2 months

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 550 nm

Temperature: 37°C

Let stand reagent and specimens at room temperature.

	Automated analyzer	Manual procedure
Reagent	250 µL	1000 µL
Standard, Controls, Specimen	5 µL	20 µL

Mix well. Let stand for 10 minutes.
Record absorbance at 550 nm (530-570) against reagent blank.

Notes:

1- Performances and stability data's have been validated on KENZA 240TX and KENZA 450TX.
2-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.
3- Applications proposal are available on request
4-Specimen blank (replace reagent by saline) or bichromatic method (2cd wavelength 600 or 700nm) is recommended for cloudy or hemolysed serum

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

With Specimen blank :
Replace Abs (Assay) in the formula by
Abs (Assay) - Abs (Specimen blank)

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 477-530.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 916-921*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-498 à 3-511*
(4) *GORNALL A. C., BARDAWILL C. J., DAVID M. M., J. Biol. Chem. 1949, 177, 751*
(5) *TIETZ N.W. Text book of clinical chemistry, 3d Ed. C.A. Curtis, E.R. Silverman L . M., Christensen R. H. (1995) p. 523-524.*
(6) *SRM: Standard Reference Material ®*