BIOLABO
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MANUFACTURER:
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TOTAL PROTEIN Biuret Method

Reagent for quantitative determination of total protein in human serum or plasma

REF 80016: R1 1 x 500 mL R2 1 x 500 mL
R3 1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

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) occuring 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 albumine 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

Vial R1 **SODIUM CHLORIDE**

Sodium chloride	75	mmol/L

Vial R2 **BIURET REAGENT**

Sodium hydroxide	370	mmol/L
Na-K Tartrate	10	mmo/L
Potassium iodide	3	mmol/L
Copper II sulfate	3	mmol/L

Before dilution: Corrosive, R35: Causes severe burns
Once diluted: Xi, R36/37/38: Irritating to eyes, respiratory system and skin.
S36/37/39: Wear suitable protective clothing, gloves and eyes/face protection

Vial R3 **STANDARD** Bovine Albumin 6 g/dL

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- Contents of vial R2 remains irritating after dilution (R34: causes burns).
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Fill to the top of the vial with demineralised water to complete content of vial R1 (NaCl) and vial R2 (Biuret). Mix by swirling. Diluted reagents are ready for use.

STABILITY AND STORAGE

On receipt, store Standard (vial R3) at 2-8°C.

- Standard (vial R3): transfer the requested quantity, well recap the vial and store at 2-8°C.
- Stored and used as described, reagents (vial R1, R2, R3) are stable in well recapped original vial, and without contamination, upon expiry date stated on the label of the kit.
- Once opened, working reagents (ready for use) are stable stored at 18-25°C and away from light at least for 6 months.

Discard any reagent if cloudy or if the absorbance of the prediluted mixture (1V/1V) of vials R1 and R2 is > 0.050 at 550 nm.

Don't use working reagents after expiry date stated on the label of the kit.

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.

INTERFERENCES (3)

Tests results with Procedure n°1:

Glucose:	No interference up to 11 g/L
Ascorbic Acid:	No interference up to 250 mg/L
Total Bilirubin:	No interference up to 550 µmol/L
Haemoglobin:	Positive interference above 150 µmol/L
Lipemia:	Positive interference above 0.150 abs (measured at 600nm)

Lipemia or hemolysis may cause falsely elevated results. It is recommended to perform a specimen blank to prevent these interferences (see § **MANUAL PROCEDURE**: Procedure n°2).
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION (6)

- Standard (vial R3) enclosed in the Kit or BIOLABO Multicalibrator, REF 95015 traceable to SRM927d.
- Or any calibrator traceable to a reference method or material.

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

It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other assayed control sera referring to the same method.
- 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 control.
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 months-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

Within run n = 20	*Low level*	*Normal level*
Mean g/dL	4.88	5.45
S.D. g/dL	0.06	0.06
C.V. %	1.2	1.2

Between run n = 20	*Low level*	*Normal level*
Mean g/dL	6.92	7.45
S.D. g/dL	0.11	0.13
C.V. %	1.6	1.7

Detection limit: approximately 0.21 g/dL

Sensitivity for 1 g/dL: approximately 0.028 Abs. at 550 nm.

Comparison study with commercially available reagent (Biuret method)

93 specimens (sera) within 3 g/dL and 11 g/dL are assayed with 2 methods

Y (g/dL) = 0.9758 x + 0.14819 r = 0,9879

LINEARITY

The assay is linear up to 10 g/dL. Above, dilute the specimen with saline solution and reassay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio

MANUAL PROCEDURE

Procedure n°1 (without Specimen blank)

Let stand reagents and specimens at room temperature.

Pipette into well identified test tubes:	Blank	Standard	Assay
Reagent R1	1,02 mL	1 mL	1 mL
Reagent R2	1 mL	1 mL	1 mL
Standard		20 µL	
Specimen			20 µL

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

Procedure n°2 (with Specimen blank)

Pipette into well identified test tubes	Blank	Specimen Blank	Standard	Assay
Reagent R1	1,02 mL	2 mL	1 mL	1 mL
Reagent R2	1 mL		1 mL	1 mL
Standard			20 µL	
Specimen		20 µL		20 µL

Mix well. Let stand for 10 minutes at room temperature.
Record absorbance at 550 nm (530-570) against reagent blank. Read specimen blank against reagent R1.

Notes:
- ✓ Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
- ✓ Specimen blank is recommended for cloudy, lipemic or hemolysed serum.
- ✓ Caution: Target values of control sera or multicalibrator may have been obtained with or without specimen blank.
- ✓ Bichromatic analyser: The 2nd wavelength is 600 or 700 nm.

CALCULATION

Calculate the result as follows:

Without specimen blank:

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

With specimen blank:

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

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 (2000) p. 916-917.*
(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 ®*

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