



BIOLABO
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U.S. PROTEIN Pyrogallol Red Method

Reagent for quantitative determination of total protein
 in human urines and C.S.F.

REF 97016 R1 2 x 113 mL R2 2 x 12 mL R3 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS

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IVD IN VITRO DIAGNOSTIC USE

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CLINICAL SIGNIFICANCE (1)

The determination of total protein in urines and in the cerebrospinal fluid (C.S.F.) is respectively used for the diagnosis of renal or central nervous system diseases. A rise in the concentration in urinary proteins is commonly seen in following cases: vigorous effort, fever or hypothermia, monoclonal gammopathies, nephropathy, diabetic nephropathy or urinary tract infection. The determination of total protein in the CSF represents a help to the diagnosis of meningitis, encephalitis, poliomyelitis, neurosyphilis, tumours of the central nervous system or cerebral haemorrhage.

PRINCIPLE (4) (7)

Fujita's method modified by Watanabe and al. Pyrogallol red combined with sodium molybdate forms a red coloured complex which absorbs at 460 nm. In an acid medium, the fixation of this complex on the amino groups of the proteins moves the absorption peak to 600 nm. The intensity of the blue staining measured at 600 nm (578-612) is proportional to the concentration of proteins in the specimen.

REAGENTS COMPOSITION

Vial R1 **BUFFER** Concentration in the test
 Sodium Molybdate 0.04 mmol/L

Vial R2 **PYROGALLOL RED**
 Methanol 10.0 %
 Pyrogallol Red 0.06 mmol/L

T+, F: Very toxic, flammable.
 R11 Highly flammable
 R39 Danger of serious irreversible effects
 R23/24/25 Toxic by inhalation, in contact with skin, and if swallowed
 S7 Keep container tightly closed
 S16 Keep away from sources of ignition. No smoking.
 S36/37 Wear suitable protective clothing and gloves
 S45 In case of accident or if you feel unwell, seek medical advice

Vial R3 **STANDARD**
 immediately (show the label of the vial)
 Bovine albumin 1.0 g/L (100 mg/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.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- 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.

REAGENT PREPARATION

Working reagent: Add the contents of vial R2 into vial R1.
 Mix swirling gently to homogenize working solution.

STABILITY AND STORAGE

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

Store reagents (vial R1 and R2) away from light at 18-25°C

- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C).
- Well capped in the original vial, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Working reagent (vial R1 + R2) is stable for 1 year when free from contamination
- Discard working reagent if cloudy or if absorbance at 600 nm is ≥ 0.600 or < 0.050 .
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Urines: Micturation or partial collection.
24 h Urines: Freshly collected urines, stored at 2-8°C.
 No preservative requested. Centrifuge 10 minutes at 3000 RPM and adjust pH at 7.0.
LCR: Freshly collected and centrifuged before analysis.

Avoid specimen containing blood.

Stability in urines:
 • Over 1 year at -20°C.

Stability in CSF:
 • up to 72 h at 2-8°C.
 • 6 months at -20°C.
 • Indefinitely at -70°C.

INTERFERENCES (3) (5)

Hemoglobin: 4 to 6 % overestimation.
Plasmion™: very overestimated results.

Pyrogallol Red shows a poor sensitivity regarding the light gamma-globulins chains.

Up until now, no drugs interferences have been described with this method.

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

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological controls



CALIBRATION (8)

- Standard enclosed in the Kit (vial R3) traceable to SRM 927d.
- Or any calibrator traceable to a reference method or a reference 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 ranges, even after using a new vial of fresh control.

QUALITY CONTROL

- Use Urinary Control Level 1 and Level 2 REF 95012
- Or controls 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 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)

Urines (micturation) < 14.0 mg/dL

24 h Urines

At rest < 80 mg/24 h

After intensive exercise < 250 mg/24 h

In CSF mg/dL

Premature 15 - 130

Newborn 40 - 120

< 1 month 20 - 80

> 1 month 15 - 40

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

PERFORMANCES CHARACTERISTICS

According to procedure n°1:

Within run N = 20	Low level	High level	Between run N = 20	Low level	High level
Mean mg/dL	13.1	57.6	Mean mg/dL	20	66
S.D.mg/dL	0.39	0.81	S.D.mg/dL	1.0	2.1
C.V. %	2.98	1.41	C.V. %	5.00	3.18

Detection limit: approximately 5 mg/dL

Comparison study with commercially available reagent:

$$y = 0.7581x + 10.45$$

$$r = 0.9777$$

LINEARITY (6)

Procedure n°1 (high sensitivity): linear up to 150 mg/dL

Procedure n°2 (low sensitivity): linear up to 600 mg/dL.

Above, dilute the specimen with saline solution and reassay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Procedure n°1:

Pipette into well identified test tubes:	Blank	Standard	Assay
Working reagent	1 mL	1 mL	1 mL
Demineralised water	20 µL		
Standard		20 µL	
Specimen (Note 2)			20 µL

Mix well. Let stand at least for 10 minutes at room temperature.
Read absorbance at 600 nm (578 - 612) against reagent blank.

Procedure n°2:

Pipette into well identified test tubes:	Blank	Standard	Assay
Working reagent	1 mL	1 mL	1 mL
Demineralised water	5 µL		
Standard		5 µL	
Specimen (Note 2)			5 µL

Mix well. Let stand at least for 10 minutes at room temperature.
Read absorbance at 600 nm (578 - 612) against reagent blank.

Notes:

- 1- After 30 minutes at room temperature, proteins may precipitate and false the result.
- 2- Specimen: Urines or CSF.
- 3- Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

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

REFERENCES

- (1) TIETZ N.W. *Text book of clinical chemistry*, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 512-530.
- (2) *Clinical Guide to Laboratory Test*, 4th Ed., N.W. TIETZ (2006) p.916-919.
- (3) YOUNG D.S., *Effect of Drugs on Clinical laboratory Tests*, 4th Ed. (1995) p.3-498 to 3-500 and 3-506 to 3-511
- (4) Watanabe N. and al, *Clin. chem.* 32/8 (1986), 1551-1554.
- (5) Le Bricon T., *Ann. Biol. Clin.* (2001),59, p.701-715
- (6) Andries J. Bakker, Baukje Jellema, *Ann. Biol. Chem.*, 36 (1999), p.163-1
- (7) Fujita Y. and Al., *Color reaction between pyrogallol red molybdenum complex and Protein. Bunseki Kagaku*, 1983, 32, E379-E386
- (8) SRM: Standard Reference Material®