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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CE

IVD IN VITRO DIAGNOSTIC USE

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 gammapathies, 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 immediately (show the label of the vial)**

Vial R3 **STANDARD**
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

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

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.

ALITY 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
< 1month	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*
Mean mg/dL	13.1	57.6
S.D.mg/dL	0.39	0.81
C.V. %	2.98	1.41

Between run N = 20	*Low level*	*High level*
Mean mg/dL	20	66
S.D.mg/dL	1.0	2.1
C.V. %	5.00	3.18

Detection limit: approximately 5 mg/dL

Comparison study with commercially available reagent:

y = 0.7581 x + 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

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(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.916-919.
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(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
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(8) SRM: Standard Reference Material ®