PROTEIN (Urine)

COD 12501 5 x 50 mL
Only for *in vitro* use in the clinical laboratory

BioSystems

PROTEIN
PYROGALLOL RED

INTENDED USE

Reagent for the measurement of protein concentration in human urine or cerebrospinal fluid. The obtained values in urine are useful as an aid in the diagnosis of proteinuria and in cerebrospinal fluid for detecting an alteration in the blood brain barrier.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

The glomeruli behave as ultrafilters for the plasma proteins. The degree to which individual proteins are normally filtered through the membrane is a function of their mass and charge, as well as of their plasma concentration.

Increased concentrations of protein in urine (proteinuria) can occur due to hemorrhage, increased glomerular permeability, defective tubular reabsorption, increased concentration in the plasma of an abnormal low-molecular-weight protein (such as immunoglobulin light chains), and abnormal secretion of protein into the urinary tract[1,2].

Proteinuria occurs in nearly all diseases of the kidney, such as nephrotic syndrome and glomerulonephritis. It can be also found in renal infarction and renal malignant tumors[3,4].

Elevated concentration of total protein in cerebrospinal fluid can be cause by high intracranial pressure (traumatic injury, brain tumors, intracerebral hemorrhage) or as a result of a bacteria or viral infection (meningitis, encephalitis, poliomyelitis)[3,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Protein in the sample reacts with pyrogallol red and molybdate in acid medium forming a coloured complex which can be measured by spectrophotometry[1,5].

CONTENTS AND COMPOSITION

A. Reagent: 5 x 50 mL. Pyrogallol red 60 μmol/L, sodium molybdate 40 μmol/L, succinate 50 mmol/L, pH 2.3, detergent.

WARNING: H226: Flammable liquid and vapour. H371: May cause damage to organs. P210: Keep away from heat/sparks/open flames/hot surfaces. – No smoking. P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P403+P235: Store in a well-ventilated place. Keep cool.

S. Protein (Urine) Standard. Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE AND STABILITY

Reagent (A): Store at 15-30ºC.

Protein (Urine) Standard (S): Store at 2-30ºC, once opened.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 2 months.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

SAMPLES

Urine collected by standard procedures. Collect a 24-hour urine specimen, measure the volume and store at 2-8ºC. Stable for 7 days at 4-8ºC[6].
Cerebrospinal fluid (CSF) collected by standard procedures. Do not use samples with blood. Stable for 6 days at 4-8ºC[6].

CALIBRATION

A reagent blank should be done every day and a calibration at least every 2 months, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Control Urine (cod. 18036 and cod. 18037) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES

Urine[3]: Less than 150 mg/24-h

Cerebrospinal fluid[3]: Children: 300-1000 mg/L

Adults: 150-450 mg/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 21.6 mg/L.
- Linearity limit: 2000 mg/L.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
711 mg/L	0.9 %	1.7 %
1454 mg/L	0.7 %	2.6 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: Bilirubin (up to 20 mg/dL) do not interfere. Hemolysis interfere. Other drugs and substances may interfere[2].

BIBLIOGRAPHY

1. Watanabe N et al. Urinary Protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 1986; 32:1551-1544.
2. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
4. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press,2001.
5. Orsonneau JL et al. An improved pyrogallol red-molybdate method for determining total urinary protein. *Clin Chem* 1989; 35:2233-2236.
6. World Health Organization (WHO). Use of anticoagulants in diagnostic laboratory investigations. Document WHO/DIL/LAB/99.1, Rev.2; 2002

TEST PARAMETERS

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: use Reagent A.

	A25	A15
GENERAL		
Name	**PROTEIN URINE**	**PROTEIN URINE**
Sample type	URI / LIQ	URI / LIQ
Analysis mode	endpoint mon.	endpoint mon.
Units	mg/L	mg/L
Turbidimetry test	no	no
Decimals	0	0
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	bichrom.	bichrom.
Main filter	600	600
Reference filter	670	670
Sample	6	6
Vol. R1	300	300
Vol. R2	-	-
Washing	1.2	1.2
Reading 1 (cycle)	21	14
Reading 2 (cycle)	-	-
Reagent 2 (cycle)	-	-
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	specific	specific
Number of calibrators	-	-
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.150	0.150
Kinetic blank limit	-	-
Linearity limit	2000	2000
Substrate depletion	-	-

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