COD 11501 4 x 50 mL	COD 11559 2 x 250 mL
STORE AT 15-30ºC	
Reagents for measurement of urine protein concentration Only for *in vitro* use in the clinical laboratory	

PROTEIN (Urine)

BioSystems
REAGENTS & INSTRUMENTS

CE

PROTEIN
PYROGALLOL RED

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[1,2].

CONTENTS

	COD 11501	COD 11559
A. Reagent	4 x 50 mL	2 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. 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 SRM 927 (National Institute of Standards and Technology, NIST).

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

STORAGE

Reagent (A): Store at 15-30ºC.

Protein (Urine) Standard (S): Store at 2-8ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 600 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC

– Analyzer, spectrophotometer or photometer able to read at 600 ± 10 nm

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[3].
Cerebrospinal fluid (CSF) collected by standard procedures. Do not use samples with blood. Stable for 6 days at 4-8ºC[3].

PROCEDURE

1. Pipette into labelled test tubes: (Notes 1, 2)

	Blank	Standard	Sample
Distilled water	20 µL	—	—
Protein (Urine) Standard (S)	—	20 µL	—
Sample	—	—	20 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and incubate for 10 minutes at 37ºC.
3. Read the absorbance (A) of the Standard and the Sample at 600 nm against the Blank. The colour is stable 30 minutes.

CALCULATIONS

The protein concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard}\ (mg/L) \times 24\ h\ urine\ volume\ (L) = C_{Sample}\ (mg/24\ h\ protein)$$

REFERENCE VALUES

Urine[4]: Less than 150 mg/24-h

Cerebrospinal fluid[4]:

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.

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 performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 26.0 mg/L.

– Linearity limit: 2000 mg/L.

– Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
696 mg/L	0.5 %	2.4 %
1427 mg/L	0.7 %	1.9 %

– 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.

– Interferences: Bilirubin (up to 20 mg/dL) do not interfere. Hemolysis interfere. Other drugs and substances may interfere[5].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

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[4,6].

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[4,6].

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)[4,6].

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

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. The analytic sensitivity can be improved by doubling the sample volume, although linearity is proportionally reduced.

BIBLIOGRAPHY

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5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
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