PROTEINS Coomassie

Colorimetric determination of Proteins in liquor and urine

CE IVD

ORDER INFORMATION

REF	Kit size
GD0730 00	4x125 ml

INDICATION

Elevated concentrations of total proteins in urine can be detected in most of renal diseases. Primary and secondary nephropathy may cause an increase of the permeability of the glomeruli or a decrease in the tubular reabsorption. Post-renal causes of proteinuria may be infections, bleedings or malignant diseases of the urinary tract. High levels of urine proteins are also correlated to other acute disorders as fever or physical and mental fatigue.
In liquor (cerebrospinal fluid, CSF) high proteins levels may be present in case of an increase of the intracranial pressure (due to cerebral tumors, intracerebral hemorrhages or trauma fever), in case of inflammations (mainly in the bacterial meningitis) and multiple sclerosis. An increase in the blood brain barrier causes an elevated CSF/serum ratio of total proteins.

METHOD PRINCIPLE

The method according Bradford is highly sensitive and allows the proteins determination in liquor and urine avoiding previous sample concentration. The chromogen reagent, containing Coomassie colouring, reacts with proteins yielding a blue colour which intensity is directly proportional to protein concentration in the sample.

COMPOSITION

REAGENT A:

Coomassie	700 µmol/l
$HClO_4$	300 mmol/l

STANDARD:	1x2 ml
Stabilized proteic solution	100 mg/dl

Verified against NIST reference material.

Preparation
Reagents are liquids ready to use.

Storage and stability
Store at 15-25 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

SAMPLES

Urine or liquor.
Stability:

	Temperature		
	20-25 °C	2-8 °C	-20 °C
Urine	1 day	2 days	
Liquor	1 day	6 days	1 year

INTERNAL QUALITY CONTROL

It is recommended to use controls with known Total Proteins concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H_2O	25 µl		
Standard	-	25 µl	-
Sample	-	-	25 µl

Mix and incubate for **2 minutes** at **room temperature** (20-25 °C). Read the absorbance (A) of the standard and samples at **620 (590-640) nm** against blank.
Colour is stable for 60 minutes.

CALCULATION OF RESULTS

Utilize the following formulas:

Liquor:

$$\text{Proteins, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

Urine:

$$\text{Proteins, mg/24h} = \frac{\text{A sample}}{\text{A standard}} \times \text{l/24} \times 1000$$

Note:
If urine has a very little level of proteins we suggest to improve sensibility of the method by pipetting 100 µl of urine as sample and 100 µl of standard diluted 1:5 (= 20 mg/dl) as calibrator. For the calculation use the concentration value of the standard of 20 mg/dl.

REFERENCE VALUES

Urine	< 15 mg/dl
Urine 24 h	< 120 mg
Liquor	< 50 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three standard solutions at low, medium and high concentrations. The obtained results are reported in the following tables:

Within-run

Standard	Mean (mg/dl)	SD	%CV
10 mg/dl	10.4	0.45	4.3
100 mg/dl	99.7	1.77	1.8
200 mg/dl	202.8	0.68	0.3

Between-run

Standard	Mean (mg/dl)	SD	%CV
10 mg/dl	10.7	0.73	6.8
100 mg/dl	100.8	1.78	1.8
200 mg/dl	203.9	4.04	2.0

Linearity
The assay is linear up to 250 mg/dl.

Sensitivity
Test sensitivity, in terms of limit of detection, is 2 mg/dl.

Interferences
Errors due to interfering substances in the urine are less than 2%.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY

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