

Reagent kit for the quantitative determination of total protein concentration in urine and liquor. Pyrogallol Red, direct colorimetric method.

Principle

Protein molecules in urine or liquor bind to the molybdate pyrogallol Red complex. The formation of the protein-dye complex causes a shift in the absorbance maximum from 467 nm to 598 nm.

Reference values

Urine total protein <140 mg/24h
Cerebrospinal liquor 80-320 mg/l (8,0-32,0 mg/dl)

It is recommended that each laboratory should assign its own normal range.

Reagents

1. Color reagent (R1)

Pyrogallol-red 60 µmol/l
Sodium molybdate 40 µmol/l
Sodium oxalate 1.04 mmol/l
Sodium benzoate 3.47 mmol/l
Methanol 100 mol/l
Succinic acid 50 mmol/l

2. Total protein ultrasensitive standard

Ready for use. For details please check the insert.

The 0,5 g/l standard available only in Cat. No.: 42051S, the 2,0 g/l standard available only in Cat. No.: 42051S2

Safety instructions:

Reagent 1:

X, Harmful
R68/20/21/22 Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed
S45 In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)

Sample

Urine. Cerebrospinal fluid.

PROCEDURE

Working reagent

The reagent are ready for use. Keep away from light.
If the absorbance of working reagent is higher than 0.25 at 578 nm the reagent can not be used.

Assay conditions

Wavelength: 598 nm
Temperature: 37 °C
Cuvette: 1 cm light path
Read against: reagent blank
Method: endpoint (increasing)

Pipette into cuvette

	Blank	Standard	Sample
Color reagent	1 ml	1 ml	1 ml
Sodium chloride (9 g/l)	20µl		
Standard		20µl	
Sample			20µl

Mix and incubate for 10 minutes at room temperature.

Calibration: (37°C, Pyrogallol Red method)

Two-point:

S1: Distilled water
S2: Total protein ultrasensitive standard (50,0 mg/dl) Cat. No.: 520715

Four-point:

S1: Distilled water
S2: Total protein ultrasensitive standard (200,0 mg/dl) Cat. No.: 520715200
ratio 1:3 dilution (50,0 mg/dl)
S3: Total protein ultrasensitive standard
ratio 1:1 dilution (100,0 mg/dl)
S4: Total protein ultrasensitive standard
without dilution (200,0 mg/dl)
Use distilled water for dilution!

Calibration frequency

Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{sample}}{A_{standard}} \times C_{standard} = C_{sample}$$

A = Absorbance
C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 2000 mg/l (200 mg/dl) if four-point calibration is used, and 1500 mg/l (150 mg/dl) if the calibration is two-point.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 1.9 mg/l (0,19 mg/dl) protein concentration at 578 nm.

Precision

	Reproducibility		
	Average conc. (mg/l)	SD	CV%
sample I	114	2.26	1.98
sample II	727	20.5	2.82

	Repeatability		
	Average conc. (mg/l)	SD	CV%
sample I	26.6	1.23	4.61
sample II	2448	12.9	0.53

Correlation

Comparative studies were done to compare our reagent with another commercial Total protein reagent.

The results from these studies are detailed below.

Correlation coefficient: $r = 0.9778$

Linear regression: $y \text{ (mg/l)} = 0.993x - 1.657$

(x= other commercial reagent, y= own reagent).

NOTE

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

N. Watanabe N. et. al.: Clin. Chem. 32: 1551. (1986)