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Protein Total in Urine/CSF

Pyrogallol red

Diagnostic reagent for quantitative in vitro determination of total protein in human urine or cerebrospinal fluid (CSF) on photometric systems

REF	Kit Size	Configuration
D03127B	1 x 1 L	Single Reagent
D03200	5 x 25 mL	Single Reagent
D79911	5 x 50 mL	Single Reagent
D0436917	9 x 65 mL	Single Reagent
DA1042	5 x 20 mL	Single Reagent
DT1042	5 x 20 mL	Single Reagent
DK0739	5 x 50 mL	Single Reagent
DE1842	5 x 20 mL	Single Reagent
DB20330	10 x 50 mL	Single Reagent

Additionally available:

D03600	1 x 3 mL	Protein Total in Urine/CSF Standard	
D08581	12 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08581SV	1 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08582	12 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2
D08582SV	1 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Colorimetric, endpoint, increasing reaction, Pyrogallol red
Shelf life	24 months from production date
Storage	2 – 8 °C
Wavelength	600 nm
Optical path	1 cm
Temperature	37°C
Sample	Urine or cerebrospinal fluid (CSF)

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of total protein in human urine or cerebrospinal fluid (CSF) on photometric systems.

DIAGNOSTIC SIGNIFICANCE [1, 2]

Elevated concentration of total protein in urine (proteinuria) can be detected in the majority of kidney diseases. Primary and secondary nephropathies may cause increased glomerular permeability or decreased tubular reabsorption. Post-renal causes of proteinuria are infections, bleedings or malignant diseases of the urinary tract. Elevated urine protein levels can also be related to other acute disorders like fever, as well as to physical or psychological stress.
In cerebrospinal fluid (CSF), elevated protein levels can be measured in case of increased intracranial pressure (due to brain tumors, intracerebral hemorrhage or traumatic injury), in inflammation, (especially in bacterial meningitis) as well as in multiple sclerosis. Increased permeability of the blood-CSF barrier is reflected in an elevated CSF/serum ratio of total protein.

TEST PRINCIPLE

Proteins form a red complex with pyrogallol red/molybdate. The absorbance is directly proportional to the protein concentration.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Pyrogallol red	60	µmol/L
Sodium molybdate	40	µmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.

REAGENT PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

Conditions:	Protect from light! Close immediately after use. Avoid contamination. Do not freeze the reagent!
Storage:	at 2 – 8 °C
Stability:	up to the indicated expiration date

WARNINGS AND PRECAUTIONS

1. Each individual blood donation used for production of the Protein Total in Urine/CSF Standard was found to be non-reactive when tested with approved methods for HBsAg, anti-HIV 1+2 and anti-HCV. As there is no possibility to exclude definitely that products derived from human blood transmit infectious agents, it is recommended to handle the standards with the same precautions used for patient specimens.
2. The Total Protein in Urine/CSF Standard contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. In very rare cases, samples of patients with gammopathy might give falsified results [7].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Use urine or cerebrospinal fluid (CSF).

Stability [3]:

In urine:	at 20 – 25 °C	1 day
	at 4 – 8 °C	7 days
	at -20°C	1 month
In cerebrospinal fluid:	at 20 – 25 °C	1 day
	at 4 – 8 °C	6 days
	at -20°C	1 year

Discard contaminated specimens. Freeze only once!

STANDARD

(Not included in the kit; has to be ordered separately)
Concentration for determination in urine: 1300 mg/L (1.3 g/L)
Concentration for determination in CSF: 1100 mg/L (1.1 g/L)
Storage: 2 – 8 °C
Stability: up to the indicated expiration date
Close immediately after use! Avoid contamination!
Do not freeze the standard!

TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Standard	Sample
Sample	-	-	20 µL
Standard	-	20 µL	-
Dist. water	20 µL	-	-
Reagent	1000 µL	1000 µL	1000 µL
Mix. Incubate for exactly 10 minutes at 37 °C and read absorbance (A) against reagent blank at 600 nm.			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

With standard:

$$\text{Total protein [mg/L]} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{Conc. Standard [mg/L]}$$

Unit Conversion
Total protein [g/dL] x 10 = Total protein [g/L]
Total protein [g/L] x 1000 = Total protein [mg/L]

QUALITY CONTROL AND CALIBRATION

All control solutions with protein total values determined by this method can be used. We recommend the Dialab urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a Protein total Standard. We recommend the Dialab **Protein Total in Urine/CSF Standard**.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE
The test has been developed to determine total protein concentrations within a range from 20 – 3000 mg/L.
If values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the results multiplied by 2. Samples with lower concentrations should be used with higher volumes (e.g. 50 µL sample + 1000 µL reagent).

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 20 mg/L.

PRECISION (at 37°C)

Intra-assay precision, n = 20	Mean [mg/L]	SD [mg/L]	CV [%]
Sample 1	178	5.23	2.94
Sample 2	450	5.10	1.14
Sample 3	1564	27.6	1.77

Inter-assay precision, n = 20	Mean [mg/L]	SD [mg/L]	CV [%]
Sample 1	170	3.94	2.32
Sample 2	449	9.68	2.16
Sample 3	1484	42.5	2.86

SPECIFICITY/INTERFERENCES

Errors due to interfering components in urine are < 2 %.
For further information on interfering substances refer to Young DS [8].

METHOD COMPARISON

A comparison between Dialab Total protein in Urine/CSF (y) and a commercially available test (x) using 69 samples gave following results: y = 1.02 x + 2.20 mg/L; r = 0.990.

TRACEABILITY

The assigned values of the standard are traceable to the standard reference material NIST SRM®-927.

EXPECTED VALUES [2, 4]

Urine	24 – 141 mg/24 h
Cerebrospinal fluid	< 500 mg/L*

* The value is an approximate guideline only.

LIMITATIONS

- Eventual Protein Total in Urine/CSF (Pyrogallol red) carry-over to reagents Bilirubin Auto Total (DCA) and UIBC (Ferene). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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