

Quantitative Determination of Protein In Urine/Csf
Only for *In Vitro* Diagnostic use
ORDER INFORMATION

REF	Cont.
MTP 25	1 X 25 ml
MTP 100	2 x 50 ml

CLINICAL SIGNIFICANCE

In healthy persons, the urine contains no protein or only a trace amount of protein; normally the glomeruli prevent passage of protein from the blood to the glomerular filtrate. Glomerular injury causes increased permeability to plasma proteins, resulting in proteinuria, which refers to the presence of protein in the urine. A persistent finding of proteinuria is the single most important indication of renal disease. Elevated concentration of protein in cerebro-spinal fluid (CSF) can be caused by infections and intracranial pressure.

PRINCIPLE

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

Protein + Pyrogallol Red-Molybdenum Complex → Protein Pyrogallol Red-Molybdenum Complex.

REAGENT COMPOSITION

Reagent I : Pyrogallol red Solution
Protein (Urine) Standard : Bovine albumin 100 mg/dL.

SAMPLE COLLECTION AND PRESERVATION

Urine collected by standard procedures. Collect a 24-hour urine specimen. Measure the volume and store at 2-8°C. Stable for 8 days. Cerebrospinal fluid (CSF) collected by standard procedures. Do not use samples with blood. Stable for 4 days at 2-8°C.

REAGENT PREPARATION

The Reagent is ready to use.

REAGENT STORAGE AND STABILITY

The reagents included are stable at 2-8°C until the expiry date stated on the labels.

REFERENCE VALUES

Urine	Less than 150 mg/24-h
Cerebrospinal fluid:	
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.

AUTOMATED PARAMETERS	
Wavelength	600 nm
Reaction Type	End Point
Cuvette	1 cm light path
Reaction Temperature	37°C
Reaction Type	Increasing
Measurement	Against Reagent Blank
Sample Volume	20µl
Reagent Volume	1000µl
Incubation	10 minutes
Blank Absorbance Limit	< 0.500 ABS
Low Normal	150 mg/L
High Normal	450 mg/L
Linearity	200 mg/L

MANUAL ASSAY PROCEDURE

PIPETTE INTO TEST TUBES

	BLANK	STD	SAMPLE
Sample	-	-	20µl
Standard	-	20µl	-
Reagent	1000µl	1000µl	1000µl

Mix & Incubate for 10 min. at 37°C. Measure absorbance of Sample (AT) and Standard (AS) against Reagent Blank at 600 nm.

CALCULATION

Urine 24 h=
 $\frac{A(\text{Sample})}{A(\text{standard})} \times 1000$ (Standard conc.) x vol. (L) urine 24 h= mg protein/24

CSF
 $\frac{A(\text{Sample})}{A(\text{standard})} \times 1000$ (Standard conc.)= mg/L protein in the sample

LINEARITY:

Upto linearity limit of 200 mg/L. If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

INTERFERENCES

The presence of hemoglobin in urine and CSF will falsely elevate results. Urine colored as a result of drugs and xanthochromic CSF require the use of a sample blank. A blank may be prepared using 20 mL of sample and 2.0 mL of deionized water and should be read at 600 nm

QUALITY CONTROL

A normal and abnormal level urine or cerebrospinal fluid control should be analyzed with each run of samples. The results should fall within plus or minus two standard deviations of the established value.

BIBLIOGRAPHY

Orsonneau JL et al. An improved Pyrogallol Red-Molybdate Method for Determining Total Urinary Protein. Clin Chem 1989 (35):2233-2236.