



MICROPROTEIN KIT (Pyrogallol Red method)

INTRODUCTION

Urinary Protein estimations serve as an important tool in the diagnosis of several disorders of the kidneys such as nephritis, nephrosis and diabetic nephropathy.

Elevated levels of protein in cerebrospinal fluid (CSF) may occur due to any lesion causing injury to cerebral tissues or blood brain barrier, viral, tuberculosis, mycotic, syphilitic or bacterial meningoencephalitis, polyneuritis, intracellular haemorrhage, degenerative disease or aseptic meningial reaction.

METHOD PRINCIPLE

Protein in the test sample form a blue-purple complex when reacted with a combination of pyrogallol red dye and molydic acid at pH 2.2. The Concentration of the protein in the sample is obtained by measuring the absorbance $\lambda = 630 \text{ nm}$

KIT CONTENTS

Reagent Name	MPR02050M
R1 - Microprotein Reagent	2 x 50 ml
R2 - Standard	2 ml

Refer standard value mentioned in the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagent is ready to use.

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light, avoid contamination.

CONCENTRATIONS IN THE TEST

Pyrogallol Red	0.067 mmol/l
Ammonium molybdate	0.026 mmol/l
Glycine Buffer	0.1 mmol/l

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 630 nm
- Thermostat at 37°C
- General laboratory equipment

URINE: Randomly collected or 24 hours collection. It is strongly recommended that a fresh sample of urine is used for the test. Since storage of sample at R.T or at 2-8°C could result in bacterial contamination it is recommended that a sample should be frozen at -20°C. If the test is to be performed later more than 24 hours.

Avoid contamination of samples from copper and iron. Centrifuge turbid samples before performing the test. Haematuria results in falsely elevated values.

CSF: Use fresh samples of CSF free from haemolysis.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Programme Sheets are available on request.

Wavelength	630nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Standard (S)	Test (T)
R1 Microprotein Reagent	1000 μl	1000 μl	1000 μl
Distilled water	20 μl		
R2 - Standard		20 μl	
Sample			20 μl

Mix well and incubate at 37°C for 10 minutes. Measure absorbance of standard and test against Blank at 630 nm within 60 minutes.

CALCULATION

Micro Protein concentration = $A(T) / A(S) \times \text{standard concentration}$

REFERENCE VALUES

Urine	21.3 - 119.6 mg/day
CSF	10 - 50 mg/dl
Urine (Random)	1-35 mg/dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available, it is recommended that known value samples be aliquoted, frozen and used as controls.

LINEARITY

The assay is linear upto 200 mg/dl. For higher values it is recommended to dilute the samples with distilled water and multiply the results by the dilution factor.

NOTES

- The reagent and sample volumes may be reduced or increased proportionally to accommodate the volume requirements of specific analyzers.
- Discard reagent if it becomes turbid and in presence of precipitates.
- R1 is photosensitive, protect reagent from strong light at all times.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

- Bradford, M.M., Anal. Biochem, 72, 248, 1976
- Fujita, Y. et al., Buneski Kagaku, 32, 379, 1983.

SYSTEM PARAMETERS

Method	End Point
Wavelength	630 nm
Zero Setting	Reagent Blank
Temperature Setting	25° C / 37° C
Incubation Temperature	37° C
Incubation Time	10 mins
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.02 ml (20 μl)
Reagent Volume	1.0 ml (1000 μl)
Standard Concentration	Refer Standard vial
Units	mg/dl
Factor	----
Reaction Slope	Increasing
Linearity	200 mg/dl



