

Manual Procedure

Cat. No. 12570 R 1 x 50 ml
For 50 test

Cat. No. 12571 R 2 x 50 ml
For 100 test

Total Microprotein

Pyrogallol red, Colorimetric method
For **CSF & Urine** protein assay

Liquid Reagent

Test Principle

Pyrogallol red is combined with molybdenum acid at a low pH. When the complex is combined with protein, a blue-purple color formed. The increase in absorbance at 600 nm is directly proportional to the protein concentration in the sample.

Concentrations in the test

Reagent R		
Pyrogallol red	120	µmol/L
Sodium molybdate	40	µmol/L
Sodium oxalate	1.04	mmol/L
Sodium benzoate	3.47	mmol/L
Ethanol	1.0	mol/L
Succinic acid sodium salt	46	mmol/L
Standard : The Concentration as indicated on vial.		

Stability of reagent

Reagent R: Liquid, ready to use.

The reagent is stable up to expiry date given on the label when stored at +2 → +8 °C.

Note: Don't use if the reagent is turbid.

Specimen collection and handling

- Urine:** Tests are performed on 24 hour samples. The urine should not be collected during periods of exercise because of its effect on micro protein concentration. Protein determinations should be performed with fresh specimens. If the test cannot be performed with fresh urine, specimens may be stored for 8 days at 2 - 8 °C, or for up to one year at - 20 °C.
- CSF:** Blood contamination should be avoided during CSF collection. If test cannot be performed immediately, specimen may be stored for up to 72 hours at 2 - 8 °C, or for six months at - 20 °C.

Procedure

Wavelength Spectrophotometer	Hg 623 nm (575 - 625 nm), 600 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point .

Standard

Microprotein STD. Cat. No. 16161

Assay

	Blank	Standard	Sample
Distilled water	20 µl	--	--
Standard	--	20 µl	--
Sample	--	--	20 µl
Reagent R	1000 µl	1000 µl	1000 µl
Mix, incubate for 8 min. at 37 °C or 12 min. at 20 - 25 °C. Read the absorbance (A) against reagent blank.			

Calculation

$$\text{Conc. Microprotein (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times \text{Conc. Standard (mg/dl)}$$

Linearity

Up to 250 mg/dl.

If the result exceeds 250 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Sensitivity

Based on an instrument absorbance resolution of 0.001, this procedure has a sensitivity of 1.0 mg/dl.

Interferences

It is recommended not to use urine specimens with added preservatives since some added preservatives such as HCl and benzoic acid have been shown to interfere in the protein assay, giving false low results.

Young, et al have reviewed a number of drugs and substances that may affect microprotein concentration.

Precautions

Protein standard contains sodium azide. Avoid contact with skin and mucous membranes. Don't ingest. It may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

Reference range

CSF

27 - 32 wk. of pregnancy	68 - 240	mg/dl
33 - 36 wk. of pregnancy	67 - 230	mg/dl
37 - 40 wk. of pregnancy	58 - 150	mg/dl
1 d. - 1 mth.	25 - 72	mg/dl
2 - 3 mth.	20 - 72	mg/dl
4 - 6 mth.	15 - 50	mg/dl
7 - 12 mth.	10 - 45	mg/dl
2 yr.	10 - 40	mg/dl
3 - 4 yr.	10 - 38	mg/dl
5 - 8 yr.	10 - 43	mg/dl
Adults	15 - 45	mg/dl

Urine

Urine Random		< 12	mg/dl
Urine / 24 hr.	men	0 - 150	mg/24 hr.
	women	27 - 93	mg/24 hr.

References

- M. Micart, Gerbautl, Clin. Chim. Acta 141, 77 - 84 (1984).
- Watanabe, N., Kamel, S., Ohkubo, A., Yamakna, M., Clin Chem 32 : 1551-554, 1986.
- Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.