TRUEchemie Micro-Proteins Test Kit (Pyrogallol Red)

for the quantitative determination of Microproteins concentration in Urine or CSF

INTENDED USE

The TRUEchemie Micro-Proteins Test Kit (Pyrogallol Red) is used for the quantitative determination of Micro proteins concentration in human Urine or CSF.

INTRODUCTION

Proteins are involved in the maintenance of the normal distribution of water between blood and the tissues and consist mainly of the albumin and globulin fractions. The measurement of low levels of urinary proteins is important in the detection of renal diseases. Proteinuria occurs in increased glomerular permeability and defective tubular reabsorption. Albuminuria is recognized as an early indicator of reversible renal damage in diabetics. The measurement of CSF proteins is used for the detection of increased permeability of the blood / brain barrier in various diseases.

PRINCIPLE

Proteins, in an acidic medium, combine with Pyrogallol Red and Molybdate to form a blue purple coloured complex. Intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Proteins + Pyrogallol Red + Molybdate —(Acidic medium)→ Blue Purple Coloured Complex

PACK SIZE

Kit size	2 x 50 ml
Cat. no.	ADX442
Kit contents	
1) Microproteins Reagent	2 x50 ml
2) Microproteins Standard (100 mg/dL)	1 x 5 ml

REAGENT COMPOSITION

1) Microproteins Reagent

Phosphate buffer : 25.00 mmol/L
Pyrogallol red : 0.12 mmol/L
Preservatives and stabilizers

2) Microprotein Standard

Micro proteins concentration : 100 mg/dL

REAGENT PREPARATION

Ready to use reagents.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The disposal of the residues has to be done as per local legal regulations.

CALIBRATION

The procedures are calibrated with the standard solution which is included with each series of tests. The absorbance is used to calculate the results.

REAGENT STORAGE & STABILITY

The components of the kit stored at 2-8°C will remain stable until the expiry date stated on the label. Do not use reagents over the expiration date. Cap the reagent bottle tightly and keep away from light to prevent contamination.

SPECIMEN COLLECTION AND STORAGE

Urine or CSF.
Microproteins in the s ample may be stable for 3 days at 2 - 8 ºC.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 630 nm

TEST PROCEDURE

Primary wavelength 630 nm
Temperature 37°C
Prewarm the reagent to reaction temperature.

	Blank (µl)	Standard (µl)	Sample (µl)
Micro protein Reagent	1000	1000	1000
Micro protein Standard	--	20	--
Sample	--	--	20

Mix well and incubate for 3 min at 37ºC or 5 min at room temperature (20 - 25 ºC). After incubation, zero the Photometer with the reagent blank. Read and record the incubated standard and samples.

$$\text{Calculation: } \frac{\text{Sample OD}}{\text{Standard OD}} \times 100 = \text{mg Microproteins / dL}$$

ESTIMATION OF TOTAL MICROPROTEIN IN URINE:

PROCEDURE:

1) Measure and record 24 hours Urine volume in litres.
2) Determine the Micro Total Protein concentration in mg/dl.
3) Convert the Micro Total Protein concentration into mg/L by multiplying with factor "10".
4) Multiply the Micro Total Protein concentration (mg/L) with 24 hrs. Urine volume collected in litres.

FORMULA:

Micro Total Protein excreted/24 hrs = Micro Total Protein concentration in mg/dl x 10 x Volume of 24 hrs. urine in litres.

EXAMPLE:

1) 24 hrs urine volume = 1.12 L
2) Urine Micro Total protein conc. determined =10 mg/dL.

Micro Total protein excreted in urine/ 24 hours = 10 x 10 x 1.12
= 112 mg/24 hours

The above example falls in normal range.

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

24 hour Urine: 28-141 mg/day
Random Urine: Up to 35 mg/dL
CSF: 10-50 mg/dL
It is strongly recommended that each laboratory establish its own normal range

PERFORMANCE CHARACTERISTICS

Sensitivity: 2 mg/dL
Linearity: Up to 250 mg/dL under the described assay conditions. If the concentration is greater than linearity (250 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	28.6	0.4	1.3
Control Level - 2	75.6	0.5	0.6

Inter-assay precision run to run (n=12)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	28.4	0.2	0.5
Control Level - 2	76.0	0.8	1.0

The reagent was tested for 12 days, using two different Microproteins concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Microproteins reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.996 and the regression equation is y=0.985x+0.968. The results of the performance characteristics depend on the analyzer used.

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. Bilirubin to a level of 20 mg/dl and Ascorbic acid to a level of 3.0 mg/dl have been found not to interfere with the assay.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEM PARAMETERS

Mode	:	End point
Std. conc.	:	100
Wave length	:	630 nm
Units	:	mg/dL
Flow cell Temp.	:	37°C
Blank	:	Reagent
Reagent volume	:	1000 µL
Sample volume	:	20 µL
Incubation	:	3 min. at 37°C
Low Normal (CSF)	:	10
High Normal (CSF)	:	50
24-hour Urine	:	28-141 mg/day
Random Urine	:	Up to 35
Sensitivity	:	2
Linearity	:	250
Reaction Slope	:	Increasing

REFERENCES

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   ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
2°C 8°C Temperature limit 2-8 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry

Do not use if package is damaged — Keep away from sunlight

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