

# Microprotein Reagent

## Benzethonium Chloride Method

### PRODUCT SUMMARY

Stability	:	Until Expiry at 2-25°C
Linear Range	:	0.05 - 2.0 g/L (50 - 2000 mg/L)
Specimen Type	:	Urine or CSF
Method	:	Turbidimetric
Reagent Preparation	:	Supplied ready to use.

**IVD**

### SYMBOLS IN PRODUCT LABELLING

<b>EC REP</b>	Authorized Representative		Temperature Limitation
<b>IVD</b>	For in vitro diagnostic use		Use by/Expiration Date
<b>LOT</b>	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
<b>REF</b>	Catalogue number		Manufactured by
	Consult instructions for use		
	Xi - Irritant		
<b>REAG 1</b>	Reagent 1	<b>REAG 2</b>	Reagent 2

### INTENDED USE

This reagent is intended for the in vitro quantitative determination of protein in urine and cerebrospinal fluid (CSF) for both manual and automated systems.

### CLINICAL SIGNIFICANCE<sup>1,2</sup>

The role of the renal system in the conservation of plasma proteins has been recognised for some time. Under normal physiological conditions small molecular weight proteins such as insulin pass through the glomeruli in relatively large amounts. Intermediate size proteins such as Transferrin and Albumin also pass through but only in relatively small amounts.

Most of these proteins are reabsorbed in the renal tubules such that normal urine contains less than 150 mg of protein per day. This also includes the protein of non serum origin normally secreted by the distal tubule (muco protein) and collecting ducts. Increased levels of urinary protein, (proteinuria) usually more than 0.15 g per 24 hours (150 mg/24 hours), almost always indicates disease.

Proteinuria may be classified as renal proteinuria or proteinuria with normal renal function. Renal proteinuria may be further classified as Glomerular or tubular proteinuria. Glomerular proteinuria is due to increased glomerular permeability (nephrotic syndrome) and may be seen in glomerular nephritis or secondary to other diseases such as diabetic nephropathy. Albumin is usually the predominant protein in the urine. Tubular proteinuria may be due to renal tubular damage from any cause especially pyelonephritis. Tubular proteinuria results in modest increases in the low molecular weight proteins if glomerular permeability is normal. Proteinuria with normal renal function may be the result of physiological increases in protein excretion or the production of abnormally large amounts of low molecular weight proteins. Increased protein excretion is seen during normal pregnancy, after strenuous exercise or following prolonged maintenance of an upright posture. Increases in low molecular weight proteins may be due to the production of Bence Jones protein, haemoglobinuria as a result of severe haemolysis and myoglobinuria as a result of severe muscle damage.

### METHODOLOGY

Methods employed for the determination of total protein in urine include dye binding, chemical and turbidimetric procedures, the latter being the most commonly employed technique<sup>3</sup>. The popularity of the turbidimetric procedures can be attributed to the simplicity of use and increased sensitivity.

The Thermo microprotein kit is a turbidimetric procedure in which benzethonium chloride is used as the protein denaturing agent. Proteins present in the urine are denatured by benzethonium chloride resulting in the formation of a fine suspension which is quantitated turbidimetrically at 405 nm. The reagent has been modified to overcome the problem of high concentration (Hook) effect, where very high concentrations of protein in urine can cause an apparent zero or low reading.<sup>4</sup>

### REAGENT COMPOSITION

#### Active Ingredients

Reagent 1:	Concentration
Carbonate Buffer	99.8 mmol/L
Sodium Chloride	140 mmol/L
EDTA	32 mmol/L

#### Reagent 2:

Benzethonium Chloride	20 g/L
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Reagents also contain surfactants and stabilisers necessary for optimum reagent performance.

**WARNING:** Do not mouth pipette. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information, please consult the Microprotein Reagent Material Safety Data Sheet.

R36 Irritating to eyes.  
S23 Do not breathe vapour.

### REAGENT PREPARATION

Reagents are supplied ready for use.

### STABILITY AND STORAGE

When stored between 2-25°C the reagents are stable until the expiration date stated on the bottle and kit box label.

### SPECIMEN COLLECTION AND HANDLING

Urine samples when stored at 4°C are stable for 2 - 3 days<sup>5</sup>. If a delay in transport to the laboratory is expected the use of a chemical preservative such as merthiolate (0.24 mmol/L) is recommended.

### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature and measuring absorbance between 405 - 415 nm.
- Analyser specific consumables, eg: samples cups
- Normal and Abnormal assayed controls.
- Urine Protein Standards such as the Thermo Microprotein Standards (Catalogue No: TR50943).

### ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

#### SYSTEM PARAMETERS

Temperature	Constant (see Note 2)
Primary Wavelength	405 nm (405 - 415 nm)
Assay Type	Turbidimetric
Direction	Increase
Sample:Reagent ratio	1:45
e.g. Sample vol	8 µL
Reagent 1 vol	300 µL
Reagent 2 vol	60 µL
Delay Time (sample + R1)	30 seconds
Incubation Time	360 seconds
Reagent Blank Limits	Low 0.00 AU
(405nm, 1cm lightpath)	High 2.00 AU
Linearity	0.05 - 2.0 g/L (50 - 2000 mg/L)

### CALCULATION OF RESULTS

Results, expressed as g/L or mg/L, are automatically calculated.

### 24 HOUR URINARY PROTEIN EXCRETION

- Measure and record the 24 hour urine volume in litres.
- Determine the protein concentration in g/L or mg/L using the above procedure.
- Multiply the protein concentration by the 24 hour urine volume. This value is the protein excretion/24 hours.

#### Example:

24 hour urine volume	=	1.12 litres
Urine protein concentration	=	0.13 g/L or 130 mg/L

#### 24 hour urine protein excretion

0.13 x 1.12	=	0.146 g/24 hours
130 x 1.12	=	146 mg/24 hours.

## NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The temperature of the reaction is not critical, however, the temperature of the spectrophotometer should be held constant between room temperature and 37°C.
3. The final absorbance should be measured within 10 minutes.
4. Unit conversion: g/L x 1000 = mg/L

## CALIBRATION

Commercially available urine protein standards such as the Thermo Microprotein Standards (Catalogue No: TR50943) should be used for calibration purposes. Thermo recommends that each run should be calibrated with at least 5 standards, referenced to NIST material and ranging in value from 0.1 to 2.4 g/L (100 to 2400 mg/L). For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.

However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

## QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

## LIMITATIONS

1. No "hook" affect was observed with samples containing protein concentrations up to a level of 60 g/L.
2. For a comprehensive review of factors affecting urine protein determination refer to the publication by Young<sup>6</sup>.
3. Haemoglobin interference was observed at a level of 20 mg/dL.
4. No interference from bilirubin up to a level of 26 µmol/L (1.5 mg/dL).

## EXPECTED VALUES

Urinary excretion of protein is normally less than 0.15 g/24 hours (150 mg/24 hours). Values above this almost always indicates disease<sup>1</sup>.

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.<sup>7</sup>

## PERFORMANCE DATA

The following data was obtained with the Microprotein Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on the specific analyser used.

## IMPRECISION

Within Run:	LEVEL I	LEVEL II	LEVEL III
Number of Samples	20	20	20
Mean (g/L / mg/L)	0.184 / 184	0.510 / 510	1.750 / 1750
SD (g/L / mg/L)	0.009 / 9	0.024 / 24	0.034 / 34
CV (%)	4.9	4.7	1.9

Between Day:	LEVEL I	LEVEL II	LEVEL III
Number of Samples	20	20	20
Mean (g/L / mg/L)	0.182 / 182	0.505 / 505	1.757 / 1757
SD (g/L / mg/L)	0.010 / 10	0.022 / 22	0.029 / 29
CV (%)	5.5	4.4	1.7

## ACCURACY

Comparison studies were carried out using another commercially available Benzethonium Chloride method as a reference. Normal and abnormal urine specimens were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:-

Number of sample pairs	63
Range of sample results	0.01 - 1.58 g/L (10 - 1580 mg/L)
Mean of reference method results	0.42 g/L (420 mg/L)
Mean of Microprotein results	0.44 g/L (440 mg/L)
Slope	1.14
Intercept	-0.04 g/L (-40 mg/L)
Correlation coefficient	0.992

## LINEARITY

When run as recommended the assay is linear to 2.0 g/L (2000 mg/L).

## SENSITIVITY

The reagent when run according to the recommended procedure is sensitive to a level of 0.05 g/L (50 mg/L).

## REFERENCES

1. Zilva JF, Pannall PR. "Plasma Proteins and Immunoglobulins" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke 1979; Chap XIV:305-29.
2. First MR. "Renal Function" in Clinical Chemistry theory, analysis and correlation. Kaplan LA, Amadeo JP (Ed). CV Mosby Co. 1984; Chap 23:418.
3. Koller A. "Total Urine Protein" in Clinical Chemistry theory, analysis and correlation Kaplan LA, Amadeo JP (Ed). CV Mosby Co. 1984; Chap 60: 1319-20.
4. Watkins I, Jenkins L. Clinical Chemistry 1987; 33:21 27-8.
5. Shephard MDS, Mazzachi RD. The Clinical Biochemist 1983; 4: 61-7.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990; 3: 296-300.
7. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.



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REF

## Reorder Information

Catalogue No.

REAG 1

REAG 2

TR50001

1 x 125 mL

1 x 25 mL