METANEPHRINES

Chromatographic – Spectrophotometric Determination
of Methanephrines
in Urine

40 tests **REF 3638**

INTENDED USE
Kit for quantitative *in vitro* determination of Metanephrines in urine.

PRINCIPLE
Metanephrines are adsorbed on a cationic resin. After washing of interfering substances, metanephrines are eluted and spectrophotometrically determined by oxidation to vanillin.

REAGENTS AND COLUMNS

Kit components:	**REF 3638**
REAGENT 1 EDTA	**1 vial**
***REAGENT 2** Alkalizer	**1 x 100 ml**
REAGENT 3 Metanephrine standard 100 mg/L	**1 x 4 ml**
REAGENT 4 Buffer	**2 x 160 ml**

WARNING: in case of any deposit on the vial bottom, shake until complete dissolution keeping the temperature at about 20-25°C.

***REAGENT 5** Eluent 4 mol/L	**2 x 170 ml**
***REAGENT 6** Oxidizing agent (powder)	**2 vials**
***REAGENT 7** Reducing agent (powder)	**2 vials**
COLUMNS Chromatographic columns	**40**
MEASURE for Reagent 1	**1**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and columns are stable up to the expiration date on the label.

EQUIPMENT REQUIRED BUT NOT SUPPLIED
- Thermostatic bath
- Spectrophotometer or photometer with 360 nm filter.

PREPARATION OF THE WORKING REAGENTS

REAGENT 6
Dissolve the contents of a vial of Reagent 6 with 6 ml of distilled water. Shake until complete dissolution.
STABILITY: at least 5 months at 2-8°C. Cap the bottle tightly closed.

REAGENT 7
Dissolve the contents of a vial of Reagent 7 with 6 ml of distilled water. Shake until complete dissolution.
STABILITY: at least 5 months at 2-8°C. Cap the bottle tightly closed.

SAMPLE
24 hour urine. Collect the urine, measure the volume and adjust to a 0.7-0.9 pH with concentrated HCl. Store in the dark at 2-8°C. Centrifuge or filter before use.
STABILITY: 1 week at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	360 nm
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	20-25°C
Method:	spectrophotometric
Linearity:	20 mg/L
Sensibility:	0.5 mg/L
C.V.:	< 5 %

PREPARATION OF THE STANDARD
Collect a sample of urine from a healthy subject, with a normal daily diuresis (about 1 liter). Adjust to a 0.7-0.9 pH as samples to check. Pour 25 ml of this urine in a conic test-tube and incubate in a hot bain-marie for 20 minutes. Cool under running water, and add in a 50 ml beaker:

"Normal" hydrolyzed urine	12.0 ml
Reagent 1	3 level measures

Mix to ease Reagent 1 dissolution. Adjust to 6.5 pH with Reagent 2, add drop by drop and keep mixing.
Add into two 10 ml test tubes:

	Working standard	"Normal" sample
Standard reagent 3	0.25 ml	---
"normal" hydrolyzed urine with 6.5 pH	5.00 ml	5.00 ml

PREPARATION OF THE SAMPLE
Pipette into a test-tube:

Sample	5.0 ml	

Incubate the test-tube in a hot bain-marie for 20 minutes. Cool the test-tube under running water and pour the contents into a 50 ml beaker. Then add:

Reagent 1	1 level measure	

Adjust to 6.5 pH with Reagent 2, add drop by drop and keep mixing.

PREPARATION OF THE COLUMN
Shake the columns upside down until complete resin re-suspension. Then leave the columns for a few minutes in vertical position to allow the resin to sediment again. Take the upper cap off and snap the bottom tip off. Let the liquid completely flow. Wash each column with 10 ml of distilled water to remove the buffer solution. Let the liquid completely flow.

CHROMATOGRAPHIC SEPARATION
Gradually pour the sample, the working standard and the "normal" sample in 3 different and labeled columns. Wash beakers with 10 ml of distilled water and pour the contents into the related columns. Discard the eluate and add in each column:

Distilled water	10.0 ml	discard the eluate
Reagent 4	7.5 ml	discard the eluate
Distilled water	10.0 ml	discard the eluate

Put the columns on 3 test-tubes of 15-20 ml and elute as it follows:

Reagent 5	7.5 ml	collect the eluate

Accurately mix the collected eluate. As metanephrines are not stable at the eluate pH, it is very important to immediately perform the colorimetric reaction.

COLORIMETRIC REACTION
Label a series of test-tubes as it follows: B/R Blank Reagent; S: Sample; B/S: Blank Sample; St: Working Standard; B/St: Blank Working Standard; N/S: "Normal" Sample; B/N/S: Blank "Normal" Sample. Pipette into labeled test-tubes:

	B/R	S	B/S	St	B/St	N/S	B/N/S
Eluate S	---	3.5 ml	3.5 ml	---	---	---	---
eluate work. St	---	---	---	3.5 ml	3.5 ml	---	---
normal S eluate	---	---	---	---	---	3.5 ml	3.5 ml
Reagent 5	3.5 ml	---	---	---	---	---	---
Reagent 7	---	---	0.1 ml	---	0.1 ml	---	0.1 ml

Accurately mix and add:

Reagent 6	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml

Accurately mix and let stand for 2 minutes at room temperature, then add:

Reagent 7	0.1 ml	0.1 ml	---	0.1 ml	---	0.1 ml	---

Accurately mix and read the absorbencies of the sample (As), the working standard (Astd), the "normal" sample (Ans), and their respective blanks at 360 nm, against the blank reagent.

CALCULATION
Metanephrines (mg/L) = [(AS-AbS)/(AStd-ABstd) -(ASn-ABns)] x 5
Metanephrines (mg/24h)= mg metanephrines/L x L of 24h urine

REFERENCE VALUES
- 24 hour urines: < 1 mg/24 hours

Each laboratory should define its own reference values.

REFERENCE
1. L. Peyrin et R. Mornex, Pathol. Biol. 16 (7-8), 447-455 (1968)

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