

# METANEPHRINES

Chromatographic – Spectrophotometric Determination  
of Methanephines  
in Urine

20 tests

REF KR08-20

## INTENDED USE

Kit for quantitative *in vitro* determination of Metanephines in urine.

## PRINCIPLE

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

## REAGENTS AND COLUMNS

Kit components:

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**REAGENT 1** EDTA 1 vial

**\*REAGENT 2** Alkalizer 1 x 40 ml

**\*REAGENT 3** Metanephine standard 100 mg/L 1 x 2 ml

**REAGENT 4** Buffer 1 x 85 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 1 x 90 ml

**\*REAGENT 6** Oxidizing agent (powder) 1 vial

**\*REAGENT 7** Reducing agent (powder) 1 vial

**COLUMNS** Chromatographic columns 20

**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:	0.4 mg/L
C.V.:	< 8 %

## PREPARATION OF THE SAMPLE

Pipette into a test-tube:

Sample	2.5 ml
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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 10 ml beaker. Then add:

Reagent 1	1 level measure
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Adjust to 6.5 pH with Reagent 2, add drop by drop and keep mixing. Centrifuge at 3000 rpm for 5 minutes

## 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 5 ml of distilled water to remove the buffer solution. Let the liquid completely flow.

## CHROMATOGRAPHIC SEPARATION

Gradually pour the sample in a labeled columns. Discard the eluate and add in each column:

Distilled water	5.0 ml	discard the eluate
Reagent 4	4.0 ml	discard the eluate
Distilled water	5.0 ml	discard the eluate

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

Reagent 5	4.0 ml	collect the eluate
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Accurately mix the collected eluate. As metanephines 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: BR Blank Reagent; S: Sample; BS: Blank Sample; St: Standard; BSt: Blank Standard. Pipette into labeled test-tubes:

	BR	BS	S	BSt	St
Eluate S	---	1.9 ml	1.9 ml	---	---
Reagent 5	1.9 ml	---	---	1.8 ml	1.8 ml
Standard (Reagent 3)	---	---	---	0.1 ml	0.1 ml
Reagent 7	---	50 µl	---	50 µl	---

Accurately mix and add:

Reagent 6	50 µl	50 µl	50 µl	50 µl	50 µl
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Accurately mix and let stand for 2 minutes at room temperature, then add:

Reagent 7	50 µl	---	50 µl	---	50 µl
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Accurately mix and read the absorbencies of the sample (As), the Standard (Astd) and their respective blanks at 360 nm, against the blank reagent.

## CALCULATION

Metanephines (mg/L) = [(AS-ABS)/(ASt-ABSt)] x 8.42

Metanephines (mg/24h) = mg metanephines/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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Issue 01 - Nov 2021



Manufactured by: **FAR** srl

Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY

Phone +39 045 6700870 - Fax +39 045 7157763

website: <http://www.fardiag.com> e-mail: [fardiag@fardiag.com](mailto:fardiag@fardiag.com)