VANILLYLMANDELIC ACID

Chromatographic – spectrophotometric determination
of 3-Methoxy-4-Hydroxymandelic Acid
(Vanillylmandelic Acid VMA) in urine

20 tests **REF KR12-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Vanillylmandelic Acid in urine.

PRINCIPLE

VMA is adsorbed on an anionic resin with buffer. After washing the interfering substances, VMA is eluted and determined spectrophotometrically by oxidation to vanillin, obtained by methaperiodate in alkaline medium.

REAGENTS AND COLUMNS

Kit components:	REF KR12-20
REAGENT 1 Phosphate buffer	**1 x 12 ml**
REAGENT 2 Sodium chloride	**1 x 110 ml**
***REAGENT 3** Carbonate potassium	**1 x 25 ml**
***REAGENT 4** Methaperiodate sodium (pre dosed)	**1 vial**
***REAGENT 5** Methasulphite sodium (pre dosed)	**1 vial**
STANDARD VMA 100 mg/L	**1 x 1 ml**

NOTICE: store sealed; close the vial immediately after use and tighten the screw cap. The solution is ethyl alcohol based, so due to evaporation the vanillylmandelic acid concentration may increase, causing false low values.

COLUMN Chromatographic columns **20**

(*) 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.

PREPARATION OF THE WORKING REAGENTS

REAGENT 4

Dissolve the contents of a vial of Reagent 4 in 15 ml of distilled water. Shake gently until complete dissolution.
STABILITY: at least 6 months at 2-8°C. Store sealed.

REAGENT 5

Dissolve the contents of a vial of Reagent 5 in 15 ml of distilled water. Shake until complete dissolution.
STABILITY: at least 6 months at 2-8°C. Store sealed.

SAMPLE

24-hour urine.
Collect the 24-hour urine, mix and measure the volume.
Store at 2-8°C. If VMA is not determined within the following day, pour 10 ml of urine in a vial, mix well and bring the pH between 4 and 5, adding 1-2 drops of glacial acetic acid. Centrifuge or filter the urine before use.
STABILITY: one week at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	360 nm
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37°C
Method:	spectrophotometric
Linearity:	125 mg/L
Sensitivity:	1.5 mg/L
Recovery:	98 ± 2 %
C.V.:	3%

PREPARATION OF THE SAMPLE

Let the reagents reach room temperature. Reagents 1 and 3 may precipitate: shake reagents well before use.
Pipette into a tube:

Urine	0.5 ml
Reagent 1	0.5 ml

Mix well and make sure the pH range is between 6.5 and 7.5, otherwise adjust it with few drops of diluted sodium hydroxide.
The tube contents will be used in the test.

PREPARATION OF THE COLUMN

Remove the cap and put it back. This operation will create a pressure inside the column, to help the liquid leak.

Wear gloves and break the bottom tip off. Let the liquid drain completely.

CHROMATOGRAPHIC SEPARATION

Pour all the contents of the tube with the sample (1 ml) into a column and let the liquid flow completely. Discard the eluate. Let the liquid drain completely. Discard the eluate.
Pipette into the column:

Distilled water	2.5 ml	discard the eluate

Put the column on a 15-20 ml test tube and pipette:

Reagent 2	1.0 ml	collect the eluate

Wait until the liquid is completely drained. Add Reagent 2 for 4 times and let the liquid flow completely each time. 5 ml of eluate of Reagent 2 is obtained.

COLORIMETRIC REACTION

Mix the collected eluate and pipette into labeled tubes:

	Blank sample	Sample	Blank reagent	Standard
Eluate	2.0 ml	2.0 ml	---	---
Reagent 2	---	---	2.0 ml	2.0 ml
Standard	---	---	---	20 µl
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 4	---	0.25 ml	0.25 ml	0.25 ml

Mix thoroughly and incubate in a thermostatic bath at 37°C for 30 minutes. Pipette:

Reagent 5	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Reagent 4	0.25 ml	---	---	---

Mix thoroughly and read the absorbances of the sample (As), the blank sample (Asb) and the standard (Astd) at 360 nm against the blank reagent.

CALCULATION

VMA (mg/L) = (As - Asb) / Astd x 10
VMA (mg/24h)= mg vanillylmandelic acid / L x L 24h urine

REFERENCE VALUES

24-hour urine:
adults 1 - 11 mg/24 hours
children 0.10 - 0.18 mg/Kg body weight/24-hours
VMA/creatinine ratio:
adults 1-8 mg VMA/g creatinine in urine
children 2-12 mg VMA/g creatinine in urine

NOTES

1 No particular restriction in the diet preceding the urine collection is required, as the blank sample subtracts dietary vanillin, which may give false high values.

2 Some drugs influence the VMA urinary excretion: - increased values result from administration of insulin, reserpine, epinephrine, norepinephrine
- decreased values result from administration of morphine, pentobarbital, chloropromazine, iproniazid.
In both cases, adjust the results as follows:
VMA (mg/L) = [(A360 S - A360 SB) - (A380 S - A380 SB)] /
(A360 ST - A380 ST) x 10
Perform the readings within 60 minutes from the end of the reaction.

3 The VMA/creatinine ratio in urine allows to perform the test on a single micturition and may give quite precise results, within certain limits, for screening purposes.
Nevertheless, the 24-hour urine is preferable and it is required when the VMA/creatinine ratio is close or lightly beyond normal values limit.

REFERENCE

1. D. Wybenga et V.J. Pileggi, “Clin. Chim. Acta”, 16 (1), 147-154 (1966)

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE

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