Manufactured for Immuno-Biological Laboratories Inc.
(IBL-America)
8201 Central Avenue, NE, Suite P
Minneapolis, MN 55432
Tel: 763-780-2955
Toll Free: 1-888-523-1246

Instructions for use

2-MET (Urine) RIA Fast Track

^{125}I – Radioimmunoassay for the determination of metanephrine and normetanephrine in urine.

For research use only, not for use in diagnostic procedures.

REF **IB88526**

Σ 2 x 100

+2 +8 °C

400 kBq

English

1. Introduction

1.1 Intended use and principle of the test

^{125}I – Radioimmunoassay for the determination of metanephrine and normetanephrine in urine. For research use only, not for use in diagnostic procedures.

First, metanephrine (metadrenaline) and normetanephrine (normetadrenaline) are acylated.
The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of ^{125}I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Determination of unknown samples is achieved by comparing their activity with a standard curve prepared with known standards.

⚠ *The anti-Metanephrine antibodies used in this test kit only recognise the biologically relevant L-forms of Metanephrine. Commercially available synthetic Metanephrine is always a mixture of the D- and L-forms. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic Metanephrine is used to enrich native samples. As only about 50% of the synthetic Metanephrine – the L-portion – will be detected by use of this kit, spiked samples will be underestimated. Therefore native samples containing solely the L-form should be used.*

1.2 Background

Metanephrine and normetanephrine are the metabolites of the catecholamines epinephrine and norepinephrine, respectively. They are metabolized to vanillylmandelic acid or excreted with the urine.
Subjects with pheochromocytoma or other tumors derived from neuroendocrine cells show elevated urinary levels of total metanephrines.
As catecholamine secretion from neuroendocrine cells might show high variations, urine samples collected over a period of 24 hours are used to average these fluctuations.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Precautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for a certain type of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents - with the exception of Precipitating Reagent – and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
(7) The radioactive material (^{125}Iodine, half life 60 days, emitting ionizing X-radiation with 28 keV and G-radiation with 35.5 keV) may be received, acquired, possessed and used only by physicians, laboratories or hospitals. In compliance with regulations, a copy of the customer's current radioisotope license must be on file with the supplier. Orders cannot be shipped until the license is received by the supplier (Radiation Protection Act of June 30, 1989).
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.

(16) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(17) The results obtained with this test kit are for research use only, not for use in diagnostic procedures.
(18) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Acylation Buffer is insufficient. As a consequence metanephrine and normetanephrine will not be acylated quantitatively.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of (nor-)metanephrine level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.

4. Materials

4.1 Content of the kit

BA D-0023 REAC-TUBES **Reaction Tubes** - Ready to use

Content: Reaction Tubes in a resealable pouch

Volume: 2 x 50 tubes

BA R-0012 ACYL-CONC **Acylation Concentrate** - Concentrated

Content: Concentrated acylation reagent

Volume: 1 x 0.5 ml/vial, pink cap

Hazards identification:

H 314 Causes severe skin burns and eye damage.

BA R-0075 ACYL-DILUENT **Acylation Diluent** - Ready to use

Content: Dimethylsulfoxide

Volume: 1 x 4 ml/vial, dark grey cap

BA R-0025 PREC-REAG **Precipitating Reagent -** Ready to use

Content: Goat anti-rabbit serum in PEG phosphate buffer

Volume: 2 x 55 ml/vial, white cap

BA R-8619 HCL **Hydrochloric Acid** - Ready to use

Content: 0.25 M hydrochloric acid, yellow coloured

Volume: 1 x 30 ml/vial, dark green cap

BA R-0120 ^{125}I ADR MN **^{125}I – Metanephrine** - Ready to use

Content: ^{125}I labeled Metanephrine, red coloured

Volume: 1 x 5.5 ml/vial, blue cap

Hazards identification:

Radioactive, activity < 200 kBq

BA R-0220 ^{125}I NAD NMN **^{125}I – Normetanephrine** - Ready to use

Content: ^{125}I labeled Normetanephrine, red coloured

Volume: 1 x 5.5 ml/vial, yellow cap

Hazards identification:

Radioactive, activity < 200 kBq

BA R-8410 AS MN **Metanephrine Antiserum** - Ready to use

Content: Rabbit anti-metanephrine antibody, blue coloured

Volume: 1 x 5.25 ml/vial, blue cap

BA R-8510 AS NMN **Normetanephrine Antiserum** - Ready to use

Content: Rabbit anti-normetanephrine antibody, yellow coloured

Volume: 1 x 5.25 ml/vial, yellow cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml		Concentration nmol/l		Volume/ Vial
			MN	NMN	MN	NMN	
BA R-8601	STANDARD A	white	0	0	0	0	12 ml
BA R-8602	STANDARD B	light yellow	20	30	101	164	4 ml
BA R-8603	STANDARD C	orange	60	90	304	491	4 ml
BA R-8604	STANDARD D	dark blue	200	300	1 014	1 638	4 ml
BA R-8605	STANDARD E	light grey	600	900	3 042	4 914	4 ml
BA R-8606	STANDARD F	black	2 000	3 000	10 140	16 380	4 ml
BA R-8651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!				4 ml
BA R-8652	CONTROL 2	dark red					4 ml

Conversion: Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)
Normetanephrine (ng/ml) x 5.46 = Normetanephrine (nmol/l)

Content: Acidic buffer with non-mercury preservatives, spiked with defined quantity of metanephrine and normetanephrine

BA R-8611 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: TRIS buffer

Volume: 1 x 30 ml/vial, white cap

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 - 3000 µl
- Polystyrene tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Temperature controlled water bath (37°C, 90°C) or similar heating device
- Suitable device for aspirating or decanting the tubes
- Gamma counter
- Vortex mixer
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Spontaneous or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, should be used.
Determine the total volume of urine excreted during 24 h for calculation of the results!
Storage: up to 5 days at 2-8 °C, for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing of the samples should be avoided.
Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents – with the exception of Precipitating Reagent - to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes accordingly. Duplicates are recommended.

⚠ *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge the tubes for 1 minute at 500 x g to spin down adhering liquids.*

6.1 Preparation of reagents

Acylation Solution

⚠ *Before preparing the Acylation Solution make sure that the Acylation Diluent (BA R-0075) has reached room temperature (≥ 20 °C) and forms a homogenous, crystal-free solution.*

Dilute the Acylation Concentrate (BA R-0012) 1 + 60 with Acylation-Diluent in a glass or polypropylene vial.

Acylation Concentrate	10 µl	20 µl	25 µl	50 µl
Acylation-Diluent	600 µl	1.2 ml	1.5 ml	3 ml

⚠ *The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!*

6.2 Preparation and acylation

Hydrolysis

1.	Pipette **25 µl** of **standards**, **controls** and **urine samples** into the respective **Reaction Tubes.**
2.	Add **250 µl Hydrochloric Acid** to **all tubes**.
3.	Mix thoroughly (vortex) and hydrolyze for **30 min** at **90 °C**.
4.	Let the **tubes** cool down to room temperature.
⚠	***For the measurement of the free metanephrine and free normetanephrine only, leave away steps 3 and 4.***

Acylation

1.	Pipette **250 µl** of **Acylation Buffer** into **all tubes**.
2.	Add **25 µl** of **Acylation Solution** *(refer to 6.1)* to **all tubes**.
3.	Mix thoroughly (vortex) and acylate for **15 min** at **RT** (20 - 25 °C).
4.	Add **1 ml water** (deionized, distilled, or ultra-pure) to **all tubes**.
⚠	The following volumes of the eluates are needed for the subsequent RIA:

Metanephrine 25 µl | **Normetanephrine 25 µl**

6.3 Metanephrine RIA

1.	Pipette **25 µl** of the **acylated Standard A** into polysterene **tubes** for the **NSB**.
2.	Pipette **25 µl** of the **acylated standards, controls** and **samples** into the respective **tubes**.
3.	Pipette **50 µl** of the **^{125}I Metanephrine** into **all tubes**.
4.	Pipette **50 µl** of **Metanephrine Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly.
5.	Cover **tubes**. Incubate for **60 min** at **37 °C.**
6.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µl** into **all tubes *(except totals)***, and mix on a vortex.
7.	Incubate for **15 min** at **2 - 8 °C**.
8.	Centrifuge for **15 min** at **3 000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant carefully *(except totals)***. Blot the tubes dry and leave them upside down for 2 minutes.
10.	**Count** all tubes for **1 min** in a gamma counter.

6.4 Normetanephrine RIA

1.	Pipette **25 µl** of the **acylated Standard A** into the polysterene **tubes** for the **NSB**.
2.	Pipette **25 µl** of the **acylated standards, controls** and **samples** into the respective **tubes**.
3.	Pipette **50 µl** of the **^{125}I Normetanephrine** into **all tubes**.
4.	Pipette **50 µl** of **Normetanephrine Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly.
5.	Cover **tubes**. Incubate for **60 min** at **37 °C.**
6.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µl** into **all tubes *(except totals)***, and mix on a vortex.
7.	Incubate for **15 min** at **2 - 8 °C**.
8.	Centrifuge for **15 min** at **3 000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant** **carefully** ***(except totals)***. Blot the tubes dry and leave them upside down for 2 minutes.
10.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

Measuring range	**Metanephrine**	**Normetanephrine**
	8 – 2 000 ng/ml	22 – 3 000 ng/ml

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The standard curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/ (B_0-NSB) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the counts are decreasing with increasing concentrations of the analyte. Counts found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations of the **samples** and **controls** can be read directly from the standard curve.

The total amount of Metanephrine and Normetanephrine excreted per day (µg/day) is calculated according to:

concentration read from the standard curve (in µg/l) x volume of urine excreted per day (in l/day)

Example
The concentration of the sample read from the curve is 125 µg/l. The amount of urine collected during 24 hours is 1.3 l. Then the amount of analyte excreted during one day would be:

125 µg/l x 1.3 l/day = 162.5 µg/day

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and have to be re-assayed.

Conversion
Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)
Normetanephrine (ng/ml) x 5.46 = Normetanephrine (nmol/l)

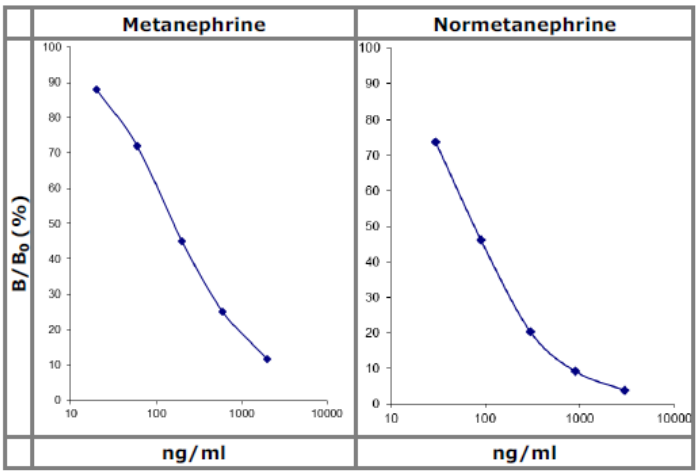
Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

	Metanephrine	**Normetanephrine**
24-hour urine	< 350 µg/day	< 600 µg/day

7.1 Quality control
It is recommended to use control samples according to national regulations. Use controls at both normal and elevated levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard curves

⚠ Examples, do not use for calculation!



8. Assay characteristics

		Metanephrine	Normetanephrine
Analytical Sensitivity	LOD	9.0 ng/ml	10.4 ng/ml
	LOB	6.2 ng/ml	7.4 ng/ml
	LOQ	10.7 ng/ml	11.4 ng/ml

	Substance	**Cross Reactivity (%)**	
		Metanephrine	Normetanephrine
Analytical Specificity (Cross Reactivity)	Derivatized Metanephrine	100	0.06
	Derivatized Normetanephrine	0.33	100
	Derivatized 3-methoxytyramine	< 0.02	0.08
	Adrenaline	0.03	< 0.01
	Noradrenaline	< 0.02	1.07
	Dopamine	< 0.02	0.01
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	< 0.02	< 0.01

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Metanephrine	1	37.4 ± 5.8	15	Metanephrine	1	24.5 ± 6.8	28
	2	67.4 ± 7.9	12		2	46.3 ± 8.8	19
	3	185 ± 12.0	7		3	127 ± 16.4	13
	4	464 ± 70.0	15		4	368 ± 56.2	15
Normetanephrine	1	71.9 ± 6.4	9	Normetanephrine	1	57.4 ± 9.1	16
	2	126 ± 10.0	8		2	109 ± 12.8	12
	3	333 ± 23.2	7		3	302 ± 34.6	12
	4	824 ± 138	17		4	893 ± 113	13

		Range (ng/ml)	Serial dilution up to	Mean (%)
Linearity	Metanephrine	58.9 – 154	1:64	109
	Normetanephrine	92.0 - 496	1:64	96

		Range (ng/ml)	Range (%)	Mean (%)
Recovery	Metanephrine	22.7 - 1343	77 - 107	93
	Normetanephrine	31.9 - 980	79 - 94	88

Method comparison versus HPLC*	Metanephrine	HPLC = 1.02 RIA – 0.3	r = 0.99; n = 21
	Normetanephrine	HPLC = 1.1 RIA - 0.3	r = 0.99; n = 21
*The concentrations were assessed using both the RIA and the HPLC method (external QC samples from UK NEQAS). The correlation between RIA and HPLC is excellent. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.			

9. References/Literature

(1) Parrott et al. Urinary corticosterone and normetanephrine levels after voluntary wheel and forced treadmill running in the db/db mouse. Journal of Diabetes Mellitus, 1(4):71-78 (2011)

(2) Petramala et al. Multiple Catecholamine-Secreting Paragangliomas: Diagnosis after Hemorrhagic Stroke in a Young Woman. Endocrine Practice, 14(3):340-346 (2008)

(3) Sato et al. Central control of bone remodeling by neuromedin U. Nature Medicine, 13:1234-1240 (2007)

For orders, please contact:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P
Minneapolis, MN 55432
Email: info@ibl-america.com
Web: www.ibl-america.com
Phone: (888) 523-1246
Fax: (763) 780-2988

⚠ ***For updated literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!