LDN Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

Instructions for use

Metanephrine Urine RIA Fast Track

REF **BA R-8400**

Σ 100 +2 +8 °C IVD CE 200 kBq

Metanephrine Urine RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Metanephrine in urine.

First, Metanephrine (Metadrenaline) is quantitatively 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. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

2. Precautions, Guidelines and Warnings

- 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.
- Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- The principles of Good Laboratory Practice (GLP) have to be followed.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- 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).
- For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
- Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- 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.
- Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
- To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- A calibrator curve must be established for each run.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- 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.
- Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- 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.
- The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Materials

4.1 Contents of the kit

REF	Symbol	Reagent	Content	Colour Code	
BA D-0023	REAC-TUBES	**Reaction Tubes**	2 x 50 tubes		ready for use
BA R-0012	ACYL-CONC	**Acylation Concentrate**	1 x 0.5 ml	white	Concentrate. Has to be diluted prior to use.
BA R-0025	PREC-REAG	**Precipitating Reagent**	1 x 55 ml	white	ready for use, goat anti-rabbit serum in PEG phosphate buffer *Mix thoroughly before use!*
BA R-0075	ACYL-DILUENT	**Acylation Diluent**	1x 4 ml	dark grey	ready for use
BA R-0120	^{125}I ADR MN	**^{125}I - Adrenaline - Metanephrine**	1 x 5.5 ml	blue	ready for use, activity < 200 kBq, red coloured
BA R-8410	AS MN	**Metanephrine Antiserum**	1 x 5.25 ml	blue	ready for use, from rabbit, blue coloured
BA R-8601	STANDARD A	**Standard A**	1 x 4 ml	white	ready for use
BA R-8602	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA R-8603	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA R-8604	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA R-8605	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA R-8606	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
BA R-8611	ACYL-BUFF	**Acylation Buffer**	1 x 30 ml	white	ready for use
BA R-8619	HCL	**Hydrochloric Acid**	1 x 30 ml	dark green	ready for use, contains 0.25 M HCl, yellow coloured
BA R-8651	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA R-8652	CONTROL 2	**Control 2**	1 x 4 ml	dark red	ready for use

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: for longer period (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 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** 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 RIA: **Metanephrine 25 µl**

6.3 Metanephrine 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 polysterene **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 for 2 minutes.
10.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Metanephrine (ng/ml=µg/l)	0	20	60	200	600	2 000
Metanephrine (nmol/l)	0	101	304	1 014	3 042	10 140
Conversion:	Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)					

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/ (B0-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).

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

The amount of analyte excreted per day (µg/day) is calculated according to:

concentration of the sample (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:

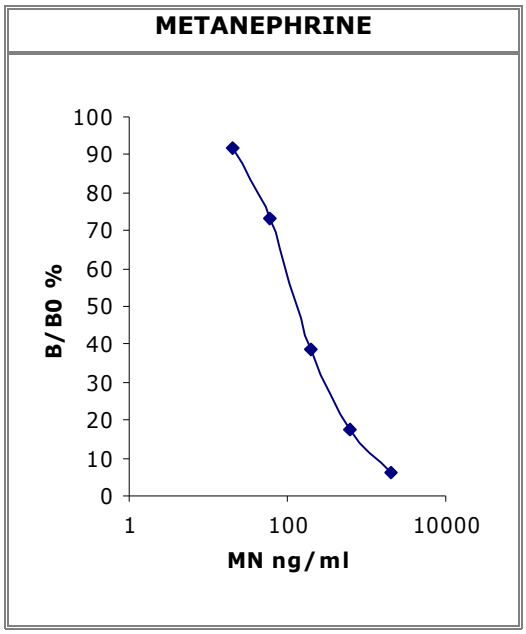
125 µg/l x 1.3 l/day = 162.5 µg/day

7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological 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 calibration curve

⚠ Example. Do not use for calculation!



8. Assay characteristics

Expected Reference Values		**Metanephrine**
	Urine	< 350 µg/day

Analytical Sensitivity (Limit of Detection)		**Metanephrine**
	Urine	13 ng/ml

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
		Metanephrine
	Derivatized Metanephrine	100
	Derivatized Normetanephrine	0.33
	Derivatized 3-methoxytyramine	0.0157
	Adrenaline	0.0314
	Noradrenaline	0.0157
	Dopamine	0.0157
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	0.0157

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Metanephrine	1	304 ± 23.1	7.6	Metanephrine	1	279 ± 26.0	9.3
	2	55.9 ± 5.9	10.6		2	43.6 ± 3.6	6.2

			Range	Serial dilution up to	Mean (%)
Linearity	Metanephrine	Urine	42 – 803 ng/ml	1:16	109

Recovery	Metanephrine Sample	Mean Creatinine mg/dl	Range Recovery(%)	Mean Recovery (%)
	1	121	73 - 121	94
	2	49	85 - 122	103

Method comparison versus HPLC*	Metanephrine	Urine	HPLC = 1.02 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.				

⚠ ***For updated literature, information about clinical significance 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!