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LDN[®]

Instructions for use

Normetanephrene Urine RIA **Fast Track**

REF

BA R-8500



IVD



200 kBq

Normetanephine Urine RIA

1. **Intended use and principle of the test**

¹²⁵I – Radioimmunoassay for the quantitative determination of Normetanephine in urine.

First, Normetanephine (Normetadrenaline) was 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 ¹²⁵I-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 (¹²⁵Iodine, half life 60 days, emitting ionizing X-radiation with 28 keV and γ -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 (NaN₃) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN₃ 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 shown 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. <i>Mix thoroughly before use!</i>
BA R-0075	ACYL-DILUENT	Acylation Diluent	1x 4 ml	dark grey	ready for use
BA R-0220	¹²⁵ I NAD NMN	¹²⁵I – Noradrenaline - Normetanephrene	1 x 5.5 ml	yellow	ready for use, activity < 200 kBq, red coloured
BA R-8510	AS MN	Normetanephrene Antiserum	1 x 5.25 ml	yellow	ready for use, from rabbit, yellow 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 ($\geq 20\text{ }^{\circ}\text{C}$) 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 $^{\circ}\text{C}$.
4.	Let the tubes cool down to room temperature.
	For the measurement of the 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 to all tubes .		
3.	Mix thoroughly (vortex) and acylate for 15 min at RT (20 - 25 $^{\circ}\text{C}$).		
4.	Add 1 ml water (deionized, distilled, or ultra-pure) to all tubes .		
	The following volumes of the eluates are needed for the RIA: <table border="1" data-bbox="199 1265 638 1310"><tr><td>Normetanephrine</td><td>25 μl</td></tr></table>	Normetanephrine	25 μl
Normetanephrine	25 μl		

6.3 Normetanephrine RIA

1.	Pipette 25 μl of the acylated Standard A into the polystyrene tubes for the NSB .
2.	Pipette 25 μl of the acylated standards, controls and samples into the respective polystyrene 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 $^{\circ}\text{C}$.
6.	Mix the chilled (2 - 8 $^{\circ}\text{C}$) 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 $^{\circ}\text{C}$.
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
Normetanephtrine (ng/ml= μ g/l)	0	30	90	300	900	3 000
Normetanephtrine (nmol/l)	0	164	491	1 638	4 914	16 380
Conversion:	Normetanephtrine (ng/ml) \times 5.46 = Normetanephtrine (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 (μ g/day) is calculated according to:

concentration of the sample (in μ g/l) \times volume of urine excreted per day (in l/day)

Example


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

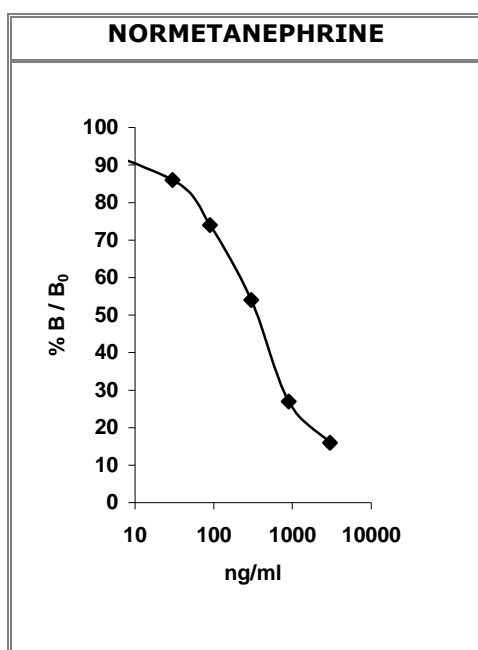
$$125 \mu\text{g/l} \times 1.3 \text{ l/day} = 162.5 \mu\text{g/day}$$

7.1 Quality control

It is recommended to use control samples according 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		Normetanephrine
	Urine	< 600 µg/day

Analytical Sensitivity (Limit of Detection)		Normetanephrine
	Urine	11 ng/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Normetanephrine
	Derivatized Metanephrine	0.06
	Derivatized Normetanephrine	100
	Derivatized 3-methoxytyramine	0.08
	Adrenaline	< 0.001
	Noradrenaline	1.07
	Dopamine	0.001
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	< 0.001

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Normetanephrine	1	189 ± 12	6.6	Normetanephrine	1	230 ± 12	5.2
	2	1,095 ± 73	6.6		2	1,596 ± 100	6.3

Linearity			Range	Serial dilution up to	Mean (%)
	Normetanephrine	Urine	105 – 2,528 ng/ml	1:16	90













Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Normetanephrine	Urine	100	86 – 111	

Method comparison versus HPLC*	Normetanephrine	Urine	HPLC = 1.1 RIA – 0.3	r = 0.99; n = 21
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*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		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!