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LDN[®]

Instructions for use

Normetanephrine Plasma RIA

Fast Track

REF

BA R-8200



IVD



200 kBq

Normetanephrine Plasma RIA

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

¹²⁵I – Radioimmunoassay for the quantitative determination of free Normetanephrine in plasma.

First, the plasma proteins are removed by precipitation. Then the normetanephrine (normetadrenaline) 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 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.



The antisera used in this test kit only recognise the biologically relevant L-forms of metanephrines. Commercially available synthetic normetanephrine or 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 metanephrines are used to enrich native samples. As only about 50% of the synthetic metanephrines, i.e. the L-portion, will be detected by use of this kit, these samples will be underestimated. Therefore only native samples should be used.

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 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 (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 expiry 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-0030	PREC-REAG	Precipitating Reagent	1 x 55 ml	yellow	ready for use, goat anti-rabbit serum in PEG phosphate buffer <i>Mix thoroughly before use!</i>
BA R-0028	EQUA-REAG	Equalizing Reagent	2 x	dark green	lyophilized
BA R-0050	ADJUST-BUFF	Adjustment Buffer	2 x 4 ml	green	ready for use
BA R-0220	¹²⁵ I NAD NMN	¹²⁵ I – Noradrenaline - Normetanephine	1 x 5.5 ml	yellow	ready for use, activity < 200 kBq, red coloured
BA R-8210	AS NMN	Normetanephine Antiserum	1 x 5.25 ml	yellow	ready for use, from rabbit, yellow coloured
BA R-8301	STANDARD A	Standard A	1 x 12 ml	white	ready for use
BA R-8302	STANDARD B	Standard B	1 x 4 ml	light yellow	ready for use
BA R-8303	STANDARD C	Standard C	1 x 4 ml	orange	ready for use
BA R-8304	STANDARD D	Standard D	1 x 4 ml	dark blue	ready for use
BA R-8305	STANDARD E	Standard E	1 x 4 ml	light grey	ready for use
BA R-8306	STANDARD F	Standard F	1 x 4 ml	black	ready for use
BA R-8312	ACYL-CONC	Acylation Concentrate	1 x 1.5 ml	dark grey	concentrated
BA R-8351	CONTROL 1	Control 1	1 x 4 ml	light green	ready for use
BA R-8352	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 25 - 500 µl; 3 ml; 10 ml
- Conical tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- For the alternative protocol with short incubation times, a shaker is needed (amplitude 3 mm; approx. 600 rpm)
- Gamma Counter
- Vortex mixer
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

EDTA- or citrate-plasma has to be used. Haemolytic and especially lipemic samples should not be used in the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer periods (up to 6 months) at -20 °C.

Repeated freezing and thawing should be avoided.

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

Equalizing Reagent


The Equalizing Reagent has to be reconstituted with 10 ml water (deionized, distilled, or ultra-pure). Reconstituted Equalizing Reagent which is not used immediately has to be frozen at -20 °C (in aliquotes) and may be thawed only once.

Acylation Solution



Pipette 80 µl Acylation Concentrate to 3 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Use immediately!

 *The Acylation Solution is stable for only 3 minutes.*

6.2 Precipitation

1.	Pipette 100 µl of standards and controls and 500 µl of plasma samples into the respective Reaction Tubes .
2.	Add 500 µl Equalizing Reagent to all tubes containing standards and controls .
3.	Add 100 µl Standard A to all tubes containing plasma samples .
4.	Mix the Reaction Tubes thoroughly (vortex) and centrifuge for 15 min at 3 000 x g .
	Take 25 µl of the clear supernatant for the Normetanephine RIA.

6.3 Normetanephine RIA (*The use of conical tubes for the RIA is highly recommended!*)

1.	Pipette 25 µl of water (deionized, distilled, or ultra-pure) into the tubes for the NSB .
2.	Pipette 25 µl of the clear supernatants of standards , controls and samples into the respective tubes .
3.	Pipette 50 µl of Adjustment Buffer into all tubes (except totals) .
4.	Pipette 25 µl Acylation Solution (refer to 6.1) into all tubes (except totals) .
	<i>The Acylation Solution is stable for maximum of only 3 minutes.</i>
5.	Mix thoroughly (vortex) and incubate for 15 min at RT (20 - 25 °C).
6.	Pipette 50 µl of Normetanephine Antiserum into all tubes (except totals and NSB) ; mix thoroughly (vortex).
7.	Incubate for 1 h at RT (20 - 25 °C).
8.	Pipette 50 µl of the ¹²⁵I Normetanephine into all tubes and mix thoroughly (vortex).
9.	Cover tubes. Incubate for 15 - 20 h (overnight) at 2 - 8 °C . <i>Alternatively incubate for 2 h at RT (20 - 25 °C) on a shaker (approx. 600 rpm).</i>
10.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 500 µl into all tubes (except totals) , and mix on a vortex.
11.	Incubate for 15 min at 2 - 8 °C .
12.	Centrifuge for 15 min at 3 000 x g , if possible in a refrigerated centrifuge.
	<i>Continue without any delay with step 13.</i>
13.	Decant or aspirate the supernatant carefully (except totals) . Blot the tubes dry and leave them upside for 2 minutes.
14.	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
Normetanephine (pg/ml)	0	48	160	480	1 600	4 800
Normetanephine (pmol/l)	0	262	874	2 620	8 740	26 200
Conversion:	Normetanephine (pg/ml) x 5.46 = Normetanephine (pmol/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 taken, is obtained by using the percentage of (B-NSB)/ (B0-NSB) measured for the standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).


Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

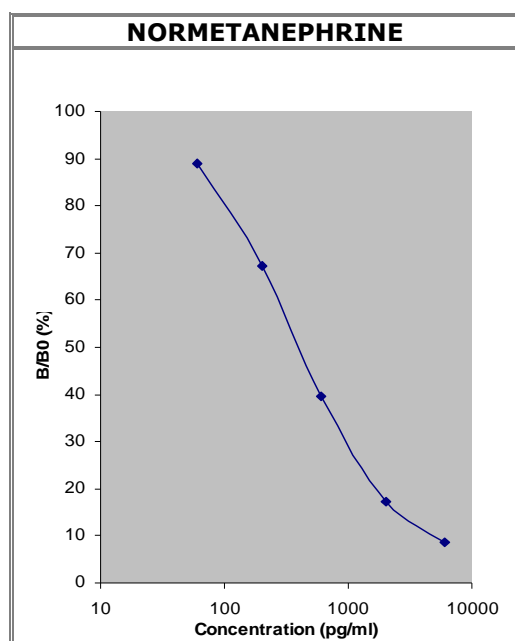
The concentrations of the **samples** and **controls** can be read directly from the standard curve.

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 commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

7.2 Typical calibration curves

 Example. Do not use for calculation!



8. Assay characteristics

Expected Reference Values		Normetanephrine
	Plasma	< 180 pg/ml

Analytical Sensitivity (Limit of Detection)		Normetanephrine
	Plasma	24 pg/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Normetanephrine
	Derivatized Metanephrine	0.08
	Derivatized Normetanephrine	100
	3-Methoxytyramin.HCl	1,74
	Adrenaline	< 0.001
	Noradrenaline	< 0.001
	Dopamin.HCl	< 0.001
	VMS	< 0.001
	HMVS	< 0.001
	L-DOPA	< 0.001
	L-Tyrosin	< 0.001
	Tyramine.HCl	< 0.001
	Normetanephrine	< 0.001
	Acetaminophen	< 0.001

Intra-Assay Precision				Inter-Assay Precision			
	Sample	Range (pg/ml)	CV (%)		Sample	Range (pg/ml)	CV (%)
Normetanephrine	1	234 ± 44	10	Normetanephrine	1	240 ± 20	8.3
	2	488 ± 43	8.7		2	518 ± 29	5.6
	3	1180 ± 93	7.9		3	1144 ± 71	6.2













Linearity			Range	Serial dilution up to	Mean (%)
	Normetanephrine	Plasma	40 - 6000	1: 149	100

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Normetanephrine	Plasma	107	95 - 119	

Method Comparison RIA vs. LC-MS/MS	Normetanephrine	Plasma	LC-MS/MS = 1.2x - 29.6	r = 0.99; n = 50
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 **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!