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LDN[®]

Instructions for use
2-CAT Urine RIA **Fast Track**

REF

BA R-7500


2 x 96


+2 ⁺⁸/_{°C}

IVD

CE

400 kBq

Adrenaline – Noradrenaline Urine RIA

1. Intended use and principle of the test

¹²⁵I– Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine) and Noradrenaline (Norepinephrine) in urine

Adrenaline (epinephrine) and noradrenaline (norepinephrine) are extracted by using a cis-diol- specific affinity gel, acylated and then converted enzymatically.

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 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 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 E-0025	WASH-CONC 50x	Wash Buffer Concentrate	1 x 20 ml	light purple	concentrated
BA R-0035	PREC-REAG	Precipitating Reagent	2 x 22 ml	orange	ready for use, goat anti-rabbit serum in PEG phosphate buffer. <i>Mix thoroughly before use!</i>
BA R-0050	ADJUST-BUFF	Adjustment Buffer	1 x 4 ml	green	ready for use
BA R-0120	¹²⁵ I ADR MN	¹²⁵I – Adrenaline - Metanephrine	1 x 5.5 ml	blue	ready for use, activity < 200 kBq, red coloured
BA R-0220	¹²⁵ I NAD NMN	¹²⁵I – Noradrenaline - Normetanephrine	1 x 5.5 ml	yellow	ready for use, activity < 200 kBq, red coloured
BA R-6210	AS NAD	Noradrenaline - Antiserum	1 x 5.25 ml	yellow	ready for use, from rabbit, yellow coloured
BA R-6611	ACYL-BUFF	Acylation Buffer	1 x 20 ml	white	ready for use
BA R-6612	ACYL-REAG	Acylation Reagent	1 x 3 ml	light red	ready for use
BA R-6614	COENZYME	Coenzyme	1 x 4 ml	purple	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	Enzyme	4 x	pink	lyophilized, contains the enzyme COMT
BA R-7110	AS ADR MN	Adrenaline – Metanephrine Antiserum	1 x 5.25 ml	blue	ready for use, from rabbit, blue coloured
BA R-7601	STANDARD A	Standard A	1 x 4 ml	white	ready for use
BA R-7602	STANDARD B	Standard B	1 x 4 ml	light yellow	ready for use
BA R-7603	STANDARD C	Standard C	1 x 4 ml	orange	ready for use
BA R-7604	STANDARD D	Standard D	1 x 4 ml	dark blue	ready for use
BA R-7605	STANDARD E	Standard E	1 x 4 ml	light grey	ready for use
BA R-7606	STANDARD F	Standard F	1 x 4 ml	black	ready for use
BA R-7617	TE-BUFF	TE Buffer	1 x 6 ml	brown	ready for use
BA R-7618	EXTRACT-PLATE 96	Extraction Plate	1 x 96 wells		coated with boronate affinity gel
BA R-7626	RELEASE-BUFF	Release Buffer	1 x 20 ml	brown	ready for use, yellow coloured, contains 0.025 M HCl
BA R-7651	CONTROL 1	Control 1	1 x 4 ml	light green	ready for use
BA R-7652	CONTROL 2	Control 2	1 x 4 ml	dark red	ready for use

4. Additional materials and equipment required but not provided in the kit


- Calibrated precision pipettes to dispense volumes between 25 – 200 µl
- Conical tubes (e.g. SARSTEDT RIA tubes) and suitable rack
- Suitable device for aspirating or decanting
- Plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Centrifuge capable of at least 3 000 x g
- Gamma counter
- Vortex mixer
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Spontaneous urine or 24-hour urine, collected in a bottle cont. 10 - 15 ml of 6 M HCl, should be used.
Storage: for a longer period (up to 6 months) at -20 °C. 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

Enzyme solution

Reconstitute the content of the vial labelled 'Enzyme' with 1 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Add 0.3 ml of Coenzyme followed by 0.7 ml of Adjustment Buffer. The total volume of the enzyme solution is 2.0 ml.


 *The enzyme solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: up to 2 months 2 - 8 °C

6.2 Derivatisation (extraction, acylation and O-methylation)

1.	Pipette 25 µl of standards, controls and urine samples into the respective wells of the Extraction Plate .
2.	Pipette 50 µl of TE Buffer into all wells.
3.	Shake 15 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).
4.	Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
5.	Pipette 100 µl of Acylation Buffer into all wells.
6.	Pipette 25 µl of Acylation Reagent into all wells.
7.	Shake 15 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).
8.	Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
9.	Pipette 150 µl of Release Buffer into all wells.
10.	Shake 5 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).
11.	Pipette 50 µl of enzyme solution (<i>prepared freshly prior to assay, refer to 6.1</i>) into all wells.
12.	Shake 30 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).  <i>Do not decant the supernatant thereafter!</i>

The following volumes of the supernatant are needed for the subsequent RIA:

Adrenaline	75 µl	Noradrenaline	25 µl
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6.3 Adrenaline RIA

1.	Pipette 75 µl of Release Buffer into the tubes for the NSB .
2.	Pipette 75 µl of the derivatized standards, controls and samples into the respective tubes .
3.	Pipette 50 µl of the ¹²⁵I Adrenaline into all tubes .
4.	Pipette 50 µl of Adrenaline-Antiserum into all tubes (except totals and NSB) ; mix thoroughly.
5.	Cover tubes. Incubate for 60 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).
6.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 200 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 <u>carefully</u> (except totals) . Blot the tubes dry and leave them upside for 2 minutes.
10.	Count all tubes for 1 min in a gamma counter.

6.4 Noradrenaline RIA

1.	Pipette 25 µl of Release Buffer into the tubes for the NSB .
2.	Pipette 25 µl of the derivatized standards, controls and samples into the respective tubes .
3.	Pipette 50 µl of the ¹²⁵I Noradrenaline into all tubes .
4.	Pipette 50 µl of Noradrenaline-Antiserum into all tubes (except totals and NSB) ; mix thoroughly
5.	Cover tubes. Incubate for 60 min at RT (20 - 25 °C) on an orbital shaker (approx. 600 rpm).
6.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 200 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 <u>carefully</u> (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

Standard	Concentration of the standards					
	A	B	C	D	E	F
Adrenaline (ng/ml)	0	1.5	4.5	15	60	240
Adrenaline (nmol/l)	0	8.19	24.6	81.9	328	1 310
Noradrenaline (ng/ml)	0	7.5	22.5	75	300	1 200
Noradrenaline (nmol/l)	0	44.3	133	443	1 773	7 092
Conversion:	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l) Noradrenaline (ng/ml) x 5.91 = Noradrenaline (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 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 the **controls** can be read directly from the standard curve.

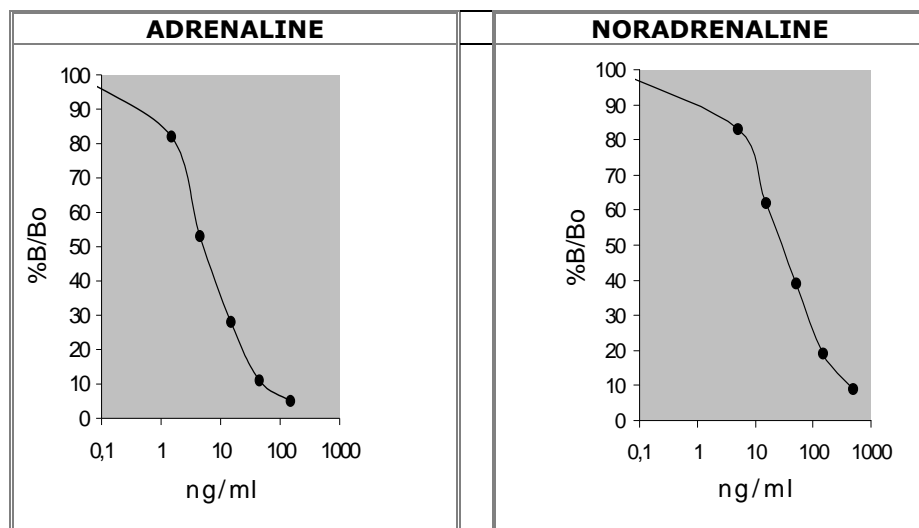
7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit controls and/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



Examples. Do not use for calculation!



8. Assay characteristics

Expected values		Adrenaline	Noradrenaline
	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD		
		Adrenaline	Noradrenaline
	Urine	0.5 ng/ml	1.7 ng/ml


Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Noradrenaline	Adrenaline
	Derivatized Adrenaline	0.14	100
	Derivatized Noradrenaline	100	0.20
	Derivatized Dopamine	0.2	< 0.0007
	Metanephrine	< 0.003	0.64
	Normetanephrine	0.48	0.0009
	3-Methoxytyramine	< 0.003	< 0.0007
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03
	Tyramine	< 0.003	< 0.0007
	Phenylalanine, Caffeinic acid, Homovanillic acid, Tyrosine, L-Dopa, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007

Precision							
Intra-Assay				Inter-Assay			
		Range (ng/ml)	CV (%)			Range (ng/ml)	CV (%)
Noradrenaline	Urine	17.8 - 126	7.2	Noradrenaline	Urine	18.6 - 117	8.9
Adrenaline	Urine	3.1 - 28.2	9.8	Adrenaline	Urine	3.4 - 25.6	8.7













Linearity			Range (ng/ml)	Serial dilution up to	Range (%)
	Noradrenaline	Urine	16.2 - 155	1:16	98 - 102
	Adrenaline	Urine	3.7 - 59	1:16	101 - 108

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Noradrenaline	Urine	102	96 - 111	
	Adrenaline	Urine	104	100 - 109	

Method comparison versus HPLC	Noradrenaline	Urine	HPLC = 1.453 x RIA +0.008	$r^2 = 0.978$
	Adrenaline	Urine	HPLC = 0.8687 x RIA +0.086	$r^2 = 0.971$

 **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!