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Instructions for use

2-CAT RIA Fast Track

REF **BA R-6500**

IVD CE 400 kBq

Adrenaline – Noradrenaline RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine) and Noradrenaline (Norepinephrine) in plasma and 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 ^{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 R-0025	PREC-REAG	**Precipitating Reagent**	2 x 55 ml	white	ready for use, goat anti-rabbit serum in PEG phosphate buffer
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 ml	green	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-0220	^{125}I NAD NMN	**^{125}I – Noradrenaline - Normetanephrine**	1 x 5.5 ml	yellow	ready for use, activity < 200 kBq, red coloured
BA R-6110	AS ADR MN	**Adrenaline – Metanephrine Antiserum**	1 x 5.25 ml	blue	ready for use, from rabbit, blue coloured
BA R-6210	AS NAD	**Noradrenaline Antiserum**	1 x 5.25 ml	yellow	ready for use, from rabbit, yellow coloured
BA R-6601	STANDARD A	**Standard A**	1 x 4 ml	white	ready for use
BA R-6602	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA R-6603	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA R-6604	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA R-6605	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA R-6606	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
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-6613	ASSAY-BUFF	**Assay Buffer**	1 x 6 ml	light grey	ready for use, contains 1 M HCl
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-6617	EXTRACT-BUFF	**Extraction Buffer**	1 x 6 ml	brown	ready for use
BA R-6618	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells		coated with boronate affinity gel
BA R-6619	HCL	**Hydrochloric Acid**	1 x 20 ml	dark green	ready for use, yellow coloured, contains 0.025 M HCl
BA R-6651	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA R-6652	CONTROL 2	**Control 2**	1 x 4 ml	dark red	ready for use
BA D-0090	FOILS	**Adhesive Foil**	1 x 4		ready for use

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

- Calibrated precision pipettes to dispense volumes between 10 - 1000 µl
- Polystyrene tubes and suitable rack
- Temperature controlled water bath, heating block or incubator (37 °C)
- Centrifuge capable of at least 3,000 x g
- Suitable device for aspirating or decanting
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Gamma counter
- Vortex mixer
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Plasma
EDTA-Plasma should be used. Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl.
Storage: for longer period (up to 6 month) 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!*

6.2 Sample preparation, extraction and acylation

1.	Pipette **10 µl** of **standards** and **controls**, **10 µl** of **urine samples** and **300 µl** of **plasma samples** into the respective wells of the **Extraction Plate**.
2.	Add **250 µl** of **water** (deionized, distilled, or ultra-pure) to the wells with **standards, controls** and **urine samples.**
3.	Pipette **50 µl** of **Assay Buffer** into all wells.
4.	Pipette **50 µl** of **Extraction Buffer** into all wells.
5.	Cover the plate with adhesive foil. Incubate for **30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
6.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.
7.	Pipette **1 ml water** (deionized, distilled, or ultra-pure) into all wells. Incubate the plate for **5 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8.	Pipette **150 µl** of **Acylation Buffer** into all wells.
9.	Pipette **25 µl** of **Acylation Reagent** into all wells.
10.	Incubate **15 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
11.	Empty the plate. Blot dry by tapping the inverted plate on absorbent material.
12.	Pipette **1 ml water** (deionized, distilled, or ultra-pure) into all wells. Incubate the plate for **5 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
13.	Pipette **150 µl** of **Hydrochloric Acid** into all wells.
14.	Cover plate with adhesive foil. Incubate **10 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ⚠ Remove the foil. ***Do not decant the supernatant thereafter!*** The following volumes of the supernatant are needed for the subsequent RIA: **Adrenaline 100 µl** **Noradrenaline 20 µl**

6.3 Adrenaline RIA

1.	Pipette **100 µl** of **Hydrochloric Acid** into the tubes for the **NSB**.
2.	Pipette **100 µl** of the **extracted standards, controls** and **samples** into the respective tubes.
3.	Pipette **25 µl** of **Enzyme Solution** (refer to 6.1*)* into all tubes ***(except totals)***.
4.	Mix thoroughly and incubate for **30 min** at **37 °C**.
5.	Pipette **50 µl** of the **^{125}I Adrenaline** into **all tubes**.
6.	Pipette **50 µl** of **Adrenaline Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly.
7.	Cover tubes. Incubate for **15 - 20 h** (overnight) at **2 - 8 °C**. ***Alternatively*** *incubate for* ***2 h at RT*** *(20 - 25 °C) on a* ***shaker*** *(approx. 600 rpm).*
8.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µl** into **all tubes *(except totals)***, and mix on a vortex.
9.	Incubate for **15 min** at **2 - 8 °C**.
10.	Centrifuge for **15 min** at **3,000 x g**, if possible in a refrigerated centrifuge.
11.	**Decant** or aspirate the **supernatant** carefully ***(except totals)***. Blot the tubes dry and leave them upside for 2 minutes.
12.	**Count** all tubes for **1 min** in a gamma counter.

6.4 Noradrenaline RIA

1.	Pipette **20 µl** of **Hydrochloric Acid** into the tubes for the **NSB**.
2.	Pipette **20 µl** of the **extracted standards, controls** and **samples** into the respective tubes.
3.	Pipette **25 µl** of **Enzyme Solution** (refer to 6.1*)* into all tubes ***(except totals)***.
4.	Mix thoroughly and incubate for **30 min** at **37 °C**.
5.	Pipette **50 µl** of the **^{125}I Noradrenaline** into **all tubes**.
6.	Pipette **50 µl** of **Noradrenaline Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly.
7.	Cover tubes. Incubate for **15 - 20 h** (overnight) at **2 - 8 °C**. ***Alternatively*** *incubate for* ***2 h at RT*** *(20 - 25 °C) on a* ***shaker*** *(approx. 600 rpm).*
8.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µl** into **all tubes *(except totals)***, and mix on a vortex.
9.	Incubate for **15 min** at **2 - 8 °C**.
10.	Centrifuge for **15 min** at **3,000 x g**, if possible in a refrigerated centrifuge.
11.	**Decant** or aspirate the **supernatant** carefully ***(except totals)***. Blot the tubes dry and leave them upside for 2 minutes.
12.	**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**
Adrenaline (ng/ml)	0	1	4	15	50	200
Adrenaline (nmol/l)	0	5.5	22	82	273	1 092
Noradrenaline (ng/ml)	0	5	20	75	250	1 000
Noradrenaline (nmol/l)	0	30	118	443	1 478	5 910
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 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).

Urine samples and controls:

The concentrations of the **urine samples** and the **Controls 1 & 2** can be read directly from the standard curve.

Calculate the 24 h excretion for each urine sample: **µg/24h = µg/l x l/24h**

Plasma samples:

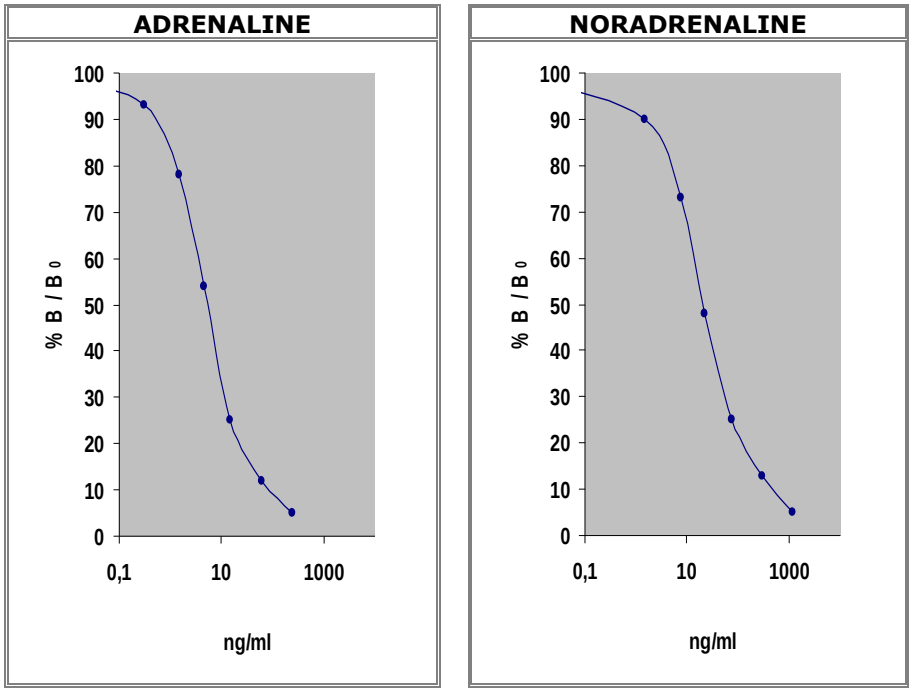
The read concentrations of the **plasma samples** have to be **divided by 30.**

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 printed on the QC-Report.

7.2 Typical calibration curve

⚠ *Examples, do not use for calculation!*



8. Assay characteristics

		Adrenaline	Noradrenaline
Expected Reference Values	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)
	Plasma	< 100 pg/ml	< 600 pg/ml

		Adrenaline	Noradrenaline
Analytical Sensitivity (Limit of Detection)	Urine	0.3 ng/ml	1.5 ng/ml
	Plasma	10 pg/ml	50 pg/ml

	Substance	Cross Reactivity (%)	
		Noradrenaline	Adrenaline
Analytical Specificity (Cross Reactivity)	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, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Noradrenaline	1	17 ± 0.66	4.0	Noradrenaline	1	22 ± 2.2	10.1
	2	58 ± 2.7	4.6		2	111 ± 6.8	6.1
Adrenaline	1	1.1 ± 0.15	13.9	Adrenaline	1	4.4 ± 0.25	5.6
	2	8.9 ± 0.40	4.6		2	21 ± 1.7	6.1

			Range	Serial dilution up to	Range (%)
Linearity	Noradrenaline	Urine	8.6 - 199 ng/ml	1:16	92 – 109
		Plasma	380 – 6,507 pg/ml	1:16	88 – 109
	Adrenaline	Urine	1.8 – 40 ng/ml	1:16	93 – 104
		Plasma	111 – 1,970 pg/ml	1:16	90 – 111

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Noradrenaline	Urine	90	81 – 100	
		Plasma	91	81 – 100	
	Adrenaline	Urine	110	100 - 133	
		Plasma	115	100 – 128	

Method Comparison versus HPLC*	Noradrenaline	Urine	HPLC = 1.23 RIA – 0.12	r = 0.99; n = 21
		Plasma	HPLC = 1.27 RIA – 0.14	r = 0.99; n = 20
	Adrenaline	Urine	HPLC = 0.95 RIA – 0.03	r = 0.99; n = 21
		Plasma	HPLC = 0.80 RIA – 0.03	r = 0.96; n = 20

*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. This means, that the RIA measure equally good when compared to the UK NEQAS HPLC data. 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!