Manufactured for Immuno-Biological Laboratories Inc.
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Instructions for use

Epinephrine (Urine) RIA

^{125}I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine) in urine.
For in-vitro diagnostic use only.

REF **IB88171**

96

+8 °C / +2

IVD

200 kBq

Adrenaline RIA

1. Intended use

^{125} I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine) in urine. For in-vitro diagnostic use only.

1.1 Principle of the test

Adrenaline (epinephrine) is 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. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
This kit contains ^{125}Iodine (half life: 60 days), emitting ionizing X- (28 kev) and G- (35.5 kev) radiations.
The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer

are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

All reagents of this testkit which contain human or animal 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.

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3. Storage and stability

The reagents should be stored at 2 - 8 °C. 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.

4.1 Contents of the kit

BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA R-0035	PREC-REAG	**Precipitating Reagent**	1 x 22 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. *Mix thoroughly before use!*
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA R-0120	^{125}I ADR MN	**^{125}I – Adrenaline - Metanephrine**	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, blue screw cap
BA R-6611	ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
BA R-6612	ACYL-REAG	**Acylation Reagent**	1 x 3 mL	ready for use
BA R-6614	COENZYME	**Coenzyme**	1 x 4 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
BA R-7110	AS ADR MN	**Adrenaline – Metanephrine Antiserum**	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-7601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-7602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-7603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-7604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-7605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-7606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-7617	TE-BUFF	**TE Buffer**	1 x 6 mL	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	ready for use, yellow coloured, contains 0.025 M HCl
BA R-7651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-7652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use

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

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1 000 µL)
- Conical tubes and suitable rack
- Suitable device for aspirating or decanting
- Plate shaker (shaking amplitude 3mm; approx. 600 rpm)
- Centrifuge capable of at least 3 000 x g
- Gamma counter, - Vortex mixer, - Absorbent material (paper towel), - Distilled water

5. Sample collection and storage

Urine

Spontaneous urine or 24-hour urine, collected in a bottle cont. 10-15 mL of 6 M HCl.
Storage: for 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 500xg to spin down adhering liquids.*

6.1 Preparation of reagents

Enzyme solution

Reconstitute the content of the vial labelled 'Enzyme' with 1 mL distilled water 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 distilled water 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**, **25 µL** of **controls,** and **25 µL** of **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 and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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** (*prepared freshly prior to assay,* refer to 6.1*)* into all wells.
12.	Shake **30 min** at **RT** (20-25°C) on an orbital **shaker** (approx. 600 rpm). ⚠ ***Do not decant the supernatant thereafter!*** The following volumes of the eluate are needed for the subsequent RIA: **Adrenaline 75 µL**

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 **^{125}I 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 minutes** 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 minutes** at **2 - 8 °C**.
8.	Centrifuge for **15 minutes** 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 minute** in a gamma counter.

7. Calculation of results

	Concentration of the standards					
Standard	**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
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (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).

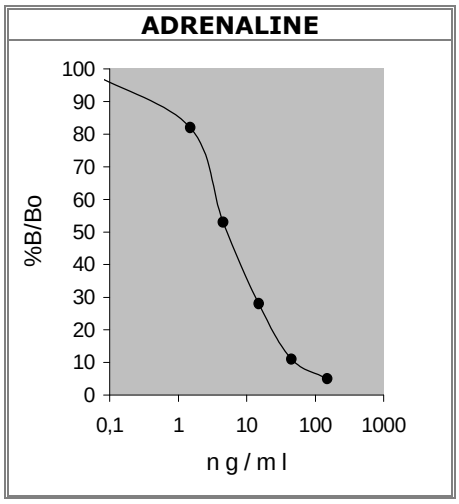
The concentrations of the samples and the controls can be read directly from the standard curve.

7.1 Quality control

It is recommended to use control samples according to state and federal 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 Reference Values		Adrenaline
	Urine	< 20 µg/day (110 nmol/day)

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

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

Precision							
Intra-Assay				**Inter-Assay**			
		Range (ng/mL)	CV (%)			Range (ng/mL)	CV (%)
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 (%)
	Adrenaline	Urine	3.7 - 59	1:16	101 - 108

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Adrenaline	Urine	104	100 - 109	

Method comparison versus HPLC	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	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
⚠	Caution	REF	Catalogue number	RUO	For research use only!