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## Instructions for use

# 3-CAT (Urine) RIA

<sup>125</sup>I – Radioimmunoassay for the quantitative determination of  
Adrenaline (Epinephrine), Noradrenaline (Norepinephrine), and Dopamine in urine.  
For in-vitro diagnostic use only.

**REF**

**IB88176**



**IVD**

**600 kBq**

## **Adrenaline – Noradrenaline - Dopamine Urine RIA**

### **1. Intended use**

<sup>125</sup>I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine), and Dopamine in urine. For in-vitro diagnostic use only.

#### **1.1 Principle of the test**

Adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine 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 <sup>125</sup>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. 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 <sup>125</sup>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.

### 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**

<b>BA E-0030</b>	WASH-CONC 50x	<b>Wash Buffer Concentrate</b>	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
<b>BA R-0035</b>	PREC-REAG	<b>Precipitating Reagent</b>	3 x 22 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. <i>Mix thoroughly before use!</i>
<b>BA R-0050</b>	ADJUST-BUFF	<b>Adjustment Buffer</b>	1 x 4 mL	ready for use
<b>BA R-0120</b>	<sup>125</sup> I ADR MN	<b><sup>125</sup>I – Adrenaline - Metanephrine</b>	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, blue screw cap
<b>BA R-0220</b>	<sup>125</sup> I NAD NMN	<b><sup>125</sup>I – Noradrenaline - Normetanephrine</b>	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, yellow screw cap
<b>BA R-0320</b>	<sup>125</sup> I-DOP	<b><sup>125</sup>I – Dopamine</b>	1 x 5.5 ml	activity < 200 kBq, ready for use, red coloured, green screw cap
<b>BA R-6210</b>	AS NAD	<b>Noradrenaline-Antiserum</b>	1 x 5.25 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
<b>BA R-6611</b>	ACYL-BUFF	<b>Acylation Buffer</b>	1 x 20 mL	ready for use
<b>BA R-6612</b>	ACYL-REAG	<b>Acylation Reagent</b>	1 x 3 mL	ready for use
<b>BA R-6614</b>	COENZYME	<b>Coenzyme</b>	1 x 4 mL	ready for use, S-adenosyl-L-methionine
<b>BA R-6615</b>	ENZYME	<b>Enzyme</b>	4 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
<b>BA R-7110</b>	AS ADR MN	<b>Adrenaline – Metanephrine Antiserum</b>	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
<b>BA R-7310</b>	AS DOP	<b>Dopamine Antiserum</b>	1 x 5.25 ml	from rabbit, ready for use, green coloured, green screw cap
<b>BA R-7601</b>	STANDARD A	<b>Standard A</b>	1 x 4 mL	ready for use
<b>BA R-7602</b>	STANDARD B	<b>Standard B</b>	1 x 4 mL	ready for use
<b>BA R-7603</b>	STANDARD C	<b>Standard C</b>	1 x 4 mL	ready for use
<b>BA R-7604</b>	STANDARD D	<b>Standard D</b>	1 x 4 mL	ready for use
<b>BA R-7605</b>	STANDARD E	<b>Standard E</b>	1 x 4 mL	ready for use
<b>BA R-7606</b>	STANDARD F	<b>Standard F</b>	1 x 4 mL	ready for use
<b>BA R-7617</b>	TE-BUFF	<b>TE Buffer</b>	1 x 6 mL	ready for use
<b>BA R-7618</b>	EXTRACT-PLATE 96	<b>Extraction Plate</b>	1 x 96 wells	coated with boronate affinity gel
<b>BA R-7626</b>	RELEASE-BUFF	<b>Release Buffer</b>	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
<b>BA R-7651</b>	CONTROL 1	<b>Control 1</b>	1 x 4 mL	ready for use
<b>BA R-7652</b>	CONTROL 2	<b>Control 2</b>	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 <b>25 µL</b> of <b>standards</b> , <b>25 µL</b> of <b>controls</b> , and <b>25 µL</b> of <b>urine samples</b> into the respective wells of the <b>Extraction Plate</b> .						
2.	Pipette <b>50 µL</b> of <b>TE Buffer</b> into all wells.						
3.	Shake <b>15 min</b> at <b>RT</b> (20-25°C) on an orbital <b>shaker</b> (approx. 600 rpm).						
4.	Discard or aspirate the contents of the wells and <b>wash</b> each well <b>3 times</b> thoroughly with <b>300 µL Wash Buffer</b> . Blot dry by tapping the inverted plate on absorbent material.						
5.	Pipette <b>100 µL</b> of <b>Acylation Buffer</b> into all wells.						
6.	Pipette <b>25 µL</b> of <b>Acylation Reagent</b> into all wells.						
7.	Shake <b>15 min</b> at <b>RT</b> (20-25°C) on an orbital <b>shaker</b> (approx. 600 rpm).						
8.	Discard or aspirate the contents of the wells and <b>wash</b> each well <b>3 times</b> thoroughly with <b>300 µL Wash Buffer</b> . Blot dry by tapping the inverted plate on absorbent material.						
9.	Pipette <b>150 µL</b> of <b>Release Buffer</b> into all wells.						
10.	Shake <b>5 min</b> at <b>RT</b> (20-25°C) on an orbital <b>shaker</b> (approx. 600 rpm).						
11.	Pipette <b>50 µL</b> of <b>enzyme solution</b> ( <i>prepared freshly prior to assay, refer to 6.1</i> ) into all wells.						
12.	Shake <b>30 min</b> at <b>RT</b> (20-25°C) on an orbital <b>shaker</b> (approx. 600 rpm). ⚠ <b>Do not decant the supernatant thereafter!</b>						
	The following volumes of the eluate are needed for the RIA:						
	<table border="1"><tr><td><b>Adrenaline</b></td><td><b>75 µL</b></td><td><b>Noradrenaline</b></td><td><b>25 µL</b></td><td><b>Dopamine</b></td><td><b>25 µL</b></td></tr></table>	<b>Adrenaline</b>	<b>75 µL</b>	<b>Noradrenaline</b>	<b>25 µL</b>	<b>Dopamine</b>	<b>25 µL</b>
<b>Adrenaline</b>	<b>75 µL</b>	<b>Noradrenaline</b>	<b>25 µL</b>	<b>Dopamine</b>	<b>25 µL</b>		

### 6.3 Adrenaline RIA

1. Pipette <b>75 µL</b> of <b>Release Buffer</b> into the tubes for the <b>NSB</b> .
2. Pipette <b>75 µL</b> of the <b>derivatized standards, controls</b> and <b>samples</b> into the respective tubes.
3. Pipette <b>50 µL</b> of the <b><sup>125</sup>I Adrenaline</b> into <b>all tubes</b> .
4. Pipette <b>50 µL</b> of <b>Adrenaline-Antiserum</b> into <b>all tubes (except totals and NSB)</b> ; mix thoroughly.
5. Cover tubes. Incubate for <b>60 minutes</b> at <b>RT (20-25°C)</b> on an orbital <b>shaker</b> (approx. 600 rpm).
6. Mix the chilled (2 - 8 °C) <b>Precipitating Reagent</b> thoroughly, pipette each <b>200 µL</b> into <b>all tubes (except totals)</b> , and mix on a vortex.
7. Incubate for <b>15 minutes</b> at <b>2 - 8 °C</b> .
8. Centrifuge for <b>15 minutes</b> at <b>3 000 x g</b> , if possible in a refrigerated centrifuge.
9. <b>Decant</b> or aspirate the <b>supernatant <u>carefully</u> (except totals)</b> . Blot the tubes dry and leave them upside for 2 minutes.
10. <b>Count</b> all tubes for <b>1 minute</b> in a gamma counter.

### 6.4 Noradrenaline RIA

1. Pipette <b>25 µL</b> of <b>Release Buffer</b> into the tubes for the <b>NSB</b> .
2. Pipette <b>25 µL</b> of the <b>derivatized standards, controls,</b> and <b>samples</b> into the respective tubes.
3. Pipette <b>50 µL</b> of the <b><sup>125</sup>I Noradrenaline</b> into <b>all tubes</b> .
4. Pipette <b>50 µL</b> of <b>Noradrenaline-Antiserum</b> into <b>all tubes (except totals and NSB)</b> ; mix thoroughly
5. Cover tubes. Incubate for <b>60 minutes</b> at <b>RT (20-25°C)</b> on an orbital <b>shaker</b> (approx. 600 rpm).
6. Mix the chilled (2 - 8 °C) <b>Precipitating Reagent</b> thoroughly, pipette each <b>200 µL</b> into <b>all tubes (except totals)</b> , and mix on a vortex.
7. Incubate for <b>15 minutes</b> at <b>2 - 8 °C</b> .
8. Centrifuge for <b>15 minutes</b> at <b>3 000 x g</b> , if possible in a refrigerated centrifuge.
9. <b>Decant</b> or aspirate the <b>supernatant <u>carefully</u> (except totals)</b> . Blot the tubes dry and leave them upside for 2 minutes.
10. <b>Count</b> all tubes for <b>1 minute</b> in a gamma counter.

### 6.5 Dopamine RIA

1. Pipette <b>25 µL</b> of <b>Release Buffer</b> into the tubes for the <b>NSB</b> .
2. Pipette <b>25 µL</b> of the <b>derivatized standards, controls,</b> and <b>samples</b> into the respective tubes.
3. Pipette <b>50 µL</b> of the <b><sup>125</sup>I Dopamine</b> into <b>all tubes</b> .
4. Pipette <b>50 µL</b> of <b>Dopamine-Antiserum</b> into <b>all tubes (except totals and NSB)</b> ; mix thoroughly.
5. Cover tubes. Incubate for <b>60 minutes</b> at <b>RT (20-25°C)</b> on an orbital <b>shaker</b> (approx. 600 rpm).
6. Mix the chilled (2 - 8 °C) <b>Precipitating Reagent</b> thoroughly, pipette each <b>200 µL</b> into <b>all tubes (except totals)</b> , and mix on a vortex.
7. Incubate for <b>15 minutes</b> at <b>2 - 8 °C</b> .
8. Centrifuge for <b>15 minutes</b> at <b>3 000 x g</b> , if possible in a refrigerated centrifuge.
9. <b>Decant</b> or aspirate the <b>supernatant <u>carefully</u> (except totals)</b> . Blot the tubes dry and leave them upside for 2 minutes.
10. <b>Count</b> all tubes for <b>1 minute</b> 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
Dopamine (ng/mL)	0	25	75	250	1 000	4 000
Dopamine (nmol/L)	0	163	490	1 633	6 530	26 120
<b>Conversion:</b>	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L) Dopamine (ng/mL) x 6.53 = Dopamine (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).

### Samples and controls:

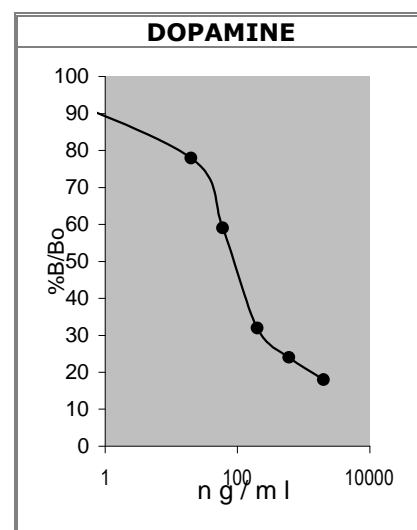
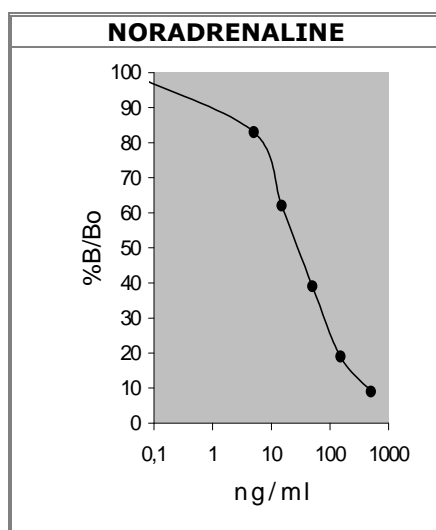
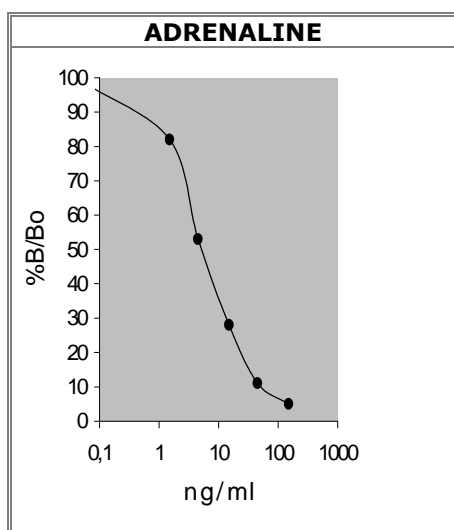
The concentrations of the samples and the controls can be read directly from the standard curve.

### 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 values		Adrenaline	Noradrenaline	Dopamine
		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	Dopamine
Urine		0.5 ng/mL	1.7 ng/mL	3 ng/mL

Analytical Specificity (Cross Reactivity)		Substance	Cross Reactivity (%)		
			Noradrenaline	Adrenaline	Dopamine
		Derivatized Adrenaline	0.14	100	0.03
		Derivatized Noradrenaline	100	0.20	0.87
		Derivatized Dopamine	0.2	< 0.0007	100
		Metanephrine	< 0.003	0.64	<0.007
		Normetanephrine	0.48	0.0009	0.008
		3-Methoxytyramine	< 0.003	< 0.0007	0.55
		3-Methoxy-4-hydroxyphenylglycol	0.01	0.03	<0.007
		Tyramine	< 0.003	< 0.0007	0.13
		Phenylalanine, Caffeinic acid, Homovanillic acid, Tyrosine, L-Dopa, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007	< 0.007

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
Dopamine	Urine	72.7 - 741	7.9	Dopamine	Urine	69.9 - 634	8.4

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		
Dopamine	Urine	43 - 688	1:16	89 - 100		







Recovery				Mean (%)	Range (%)	% Recovery after spiking
		Noradrenaline	Urine	102	96 - 111	
Adrenaline	Urine	104	100 - 109			
Dopamine	Urine	93	84 - 100			

Method Comparison versus HPLC					
		Noradrenaline	Urine	HPLC = 1.453 x RIA +0.008	r <sup>2</sup> = 0.978
Adrenaline	Urine	HPLC = 0.8687 x RIA +0.086	r <sup>2</sup> = 0.971		
Dopamine	Urine	HPLC = 0.7387 x RIA +0.370	r <sup>2</sup> = 0.898		

 **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	<b>LOT</b>	Batch code	<b>IVD</b>	For in-vitro diagnostic use only!
	Consult instructions for use	<b>CONT</b>	Content	<b>CE</b>	CE labelled
	Caution	<b>REF</b>	Catalogue number	<b>RUO</b>	For research use only!

**Manufactured for:**

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