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Instructions for use

3-CAT RIA

^{125}I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine) and Dopamine in plasma and urine.

For *in-vitro* diagnostic use only.

REF **IB88166**

Σ 3 x 96

+2 +8 °C

IVD

600 kBq

1. Introduction

1.1 Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine) and Dopamine in plasma and urine. For *in-vitro* diagnostic use only.

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 ^{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 standard curve prepared with known standards.

1.2 Clinical application

In humans the catecholamines adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are neurotransmitters of the sympathetic nervous system and are involved in many physiological processes. The sympathetic nervous system sets the body to a heightened state of alert, also called as the body's fight-or-flight response.
In the human body the catecholamines and their metabolites indicate the adaption of the body to acute and chronic stress.
Next to the metanephrine/normetanephrine the catecholamines are important for the diagnosis and the follow-up of tumors of the sympathoadrenal system like the pheochromocytoma. The quantitative determination of catecholamines in urine is preferred for the diagnosis of these tumors, whereas the determination of catecholamines in plasma is medically sensible for the localization of the tumor and for function testing. Values above the cut-off can provide an indication for neuroendocrine tumors.
However, in literature various diseases like hypertension, cardiovascular diseases, schizophrenia and manic depression are described with different levels of catecholamines.
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point "Procedural cautions, guidelines and warnings". Any laboratory result is only a part of the total clinical picture of the patient.
Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient it can be used for therapeutic consequences.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Precautions, guidelines and warnings

(1) 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.
(2) This assay was validated for certain types of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents - with the exception of Precipitating Reagent – and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
(7) 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).
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) 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.
(10) Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.

(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(14) 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.
(15) 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.
(16) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(17) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence (e.g. medication before a scheduled surgery) but have to be correlated to other diagnostic tests and clinical observations.
(18) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Extraction Buffer is insufficient. As a consequence catecholamines will not be extracted adequately.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of catecholamine level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.

4. Materials

4.1 Content of the kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Content: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA R-0025 PREC-REAG **Precipitating Reagent -** Ready to use
Content: Goat anti-rabbit serum in PEG phosphate buffer
Volume: 3 x 55 ml/vial, white cap

BA R-6618 EXTRACT-PLATE 48 **Extraction Plate** - Ready to use
Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch

BA R-0050 ADJUST-BUFF **Adjustment Buffer** - Ready to use
Content: TRIS buffer
Volume: 2 x 4 ml/vial, green cap

BA R-0120 ^{125}I ADR MN **^{125}I – Adrenaline** - Ready to use
Content: ^{125}I labeled Adrenaline, red coloured
Volume: 1 x 5.5 ml/vial, blue cap
Hazards identification: 

Radioactive, activity < 200 kBq

BA R-0220 ^{125}I NAD NMN **^{125}I – Noradrenaline** - Ready to use

Content: ^{125}I labeled Noradrenaline, red coloured

Volume: 1 x 5.5 ml/vial, yellow cap

Hazards identification: 

Radioactive, activity < 200 kBq

BA R-0320 ^{125}I DOP **^{125}I – Dopamine** - Ready to use

Content: ^{125}I labeled Dopamine, red coloured

Volume: 1 x 5.5 ml/vial, dark green cap

Hazards identification: 

Radioactive, activity < 200 kBq

BA R-6110 AS ADR MN **Adrenaline Antiserum** - Ready to use

Content: Rabbit anti- Adrenaline antibody, blue coloured

Volume: 1 x 5.25 ml/vial, blue cap

BA R-6210 AS NAD **Noradrenaline Antiserum** - Ready to use

Content: Rabbit anti- Noradrenaline antibody, yellow coloured

Volume: 1 x 5.25 ml/vial, yellow cap

BA R-6310 AS DOP **Dopamine Antiserum** - Ready to use

Content: Rabbit anti- Dopamine antibody, green coloured

Volume: 1 x 5.25 ml/vial, dark green cap

BA R-6611 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: Buffer with light alkaline pH

Volume: 1 x 20 ml/vial, white cap

BA R-6612 ACYL-REAG **Acylation Reagent** - Ready to use

Content: Acylation reagent in DMF and DMSO

Volume: 1 x 3 ml/vial, light red cap

Hazards identification: 

H225 Highly flammable liquid and vapour.
H360 May damage fertility or the unborn child.
H319 Causes serious eye irritation.

BA R-6613 ASSAY-BUFF **Assay Buffer** - Ready to use

Content: 1M hydrochloric acid and a non-mercury preservative

Volume: 1 x 6 ml/vial, light grey cap

BA R-6614 COENZYME **Coenzyme** - Ready to use

Content: S-adenosyl-L-methionine

Volume: 1 x 4 ml/vial, purple cap

BA R-6615 ENZYME **Enzyme** - Lyophilized

Content: Catechol-O-methyltransferase

Volume: 6 vials, pink cap

BA R-6617 EXTRACT-BUFF **Extraction Buffer** - Ready to use

Content: Buffer containing carbonate

Volume: 1 x 6 ml/vial, brown cap

BA R-6619 HCL **Hydrochloric Acid** - Ready to use

Content: 0.025 M Hydrochloric Acid, yellow coloured

Volume: 1 x 20 ml/vial, dark green cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/ Cap	Concentration ng/ml			Concentration nmol/l			Volume/ Vial
			ADR	NAD	DOP	ADR	NAD	DOP	
BA R-6601	STANDARD A	white	0	0	0	0	0	0	4 ml
BA R-6602	STANDARD B	light yellow	1	5	10	5.5	30	65	4 ml
BA R-6603	STANDARD C	orange	4	20	40	22	118	261	4 ml
BA R-6604	STANDARD D	dark blue	15	75	150	82	443	980	4 ml
BA R-6605	STANDARD E	light grey	50	250	500	273	1 478	3 265	4 ml
BA R-6606	STANDARD F	black	200	1 000	2 000	1 092	5 910	13 060	4 ml
BA R-6609	STANDARD A/B	light purple	-	-	4.5	-	-	29	4 ml
BA R-6651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!						4 ml
BA R-6652	CONTROL 2	dark red							4 ml

Conversion: 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)

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of adrenaline, noradrenaline, and dopamine

⚠ **for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

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

Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant (e.g. Monovette™ or Vacuette™ for plasma) and centrifuged at room temperature immediately after collection.
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.
Repeated freezing and thawing should be avoided.

Urine

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.
If 24-hour urine is used please record the total volume of the collected urine. If the percentage of the final concentration of acid is too high, the buffer capacity of the Extraction Buffer is insufficient. As a consequence catecholamines will not be extracted adequately.
Storage: up to 48 hours at 2 - 8 °C, up to 24 hours at room temperature, for longer periods (up to 6 month) at -20 °C. Repeated freezing and thawing should be avoided.
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

⚠ **for the determination of **dopamine in plasma** the additional **Standard A/B** is mandatory!*

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**
Dopamine (standards + urine)	**10 µl**	**Dopamine (plasma)**	**25 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 down 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 down for 2 minutes.
12.	**Count** all tubes for **1 min** in a gamma counter.

6.5 Dopamine RIA

⚠ **for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

1.	Pipette **10 µl** of **Hydrochloric Acid** into the tubes for the **NSB**.
2.	Pipette **10 µl** of the extracted **standards, 10 µl** of the extracted **controls, 10 µl** of the extracted **urine samples** and **25 µl** of the extracted **plasma 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 Dopamine** into **all tubes**.
6.	Pipette **50 µl** of **Dopamine 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 down for 2 minutes.
12.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

		Adrenaline	Noradrenaline	Dopamine
Measuring range	Urine	0.5 - 200 ng/ml	1.4 – 1 000 ng/ml	3.8 – 2 000 ng/ml
	Plasma	24 – 6 667 pg/ml	54 – 33 333 pg/ml	39 – 26 667 pg/ml

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The standard curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/ (B_0-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).

⚠ *This assay is a competitive assay. This means: the counts are decreasing with increasing concentrations of the analyte. Counts found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

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
Adrenaline and Noradrenaline:
The read concentrations of the **plasma samples** have to be **divided by 30.**
Dopamine:
The read concentrations of the **plasma samples** have to be **divided by 75.**

Conversion
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)

Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

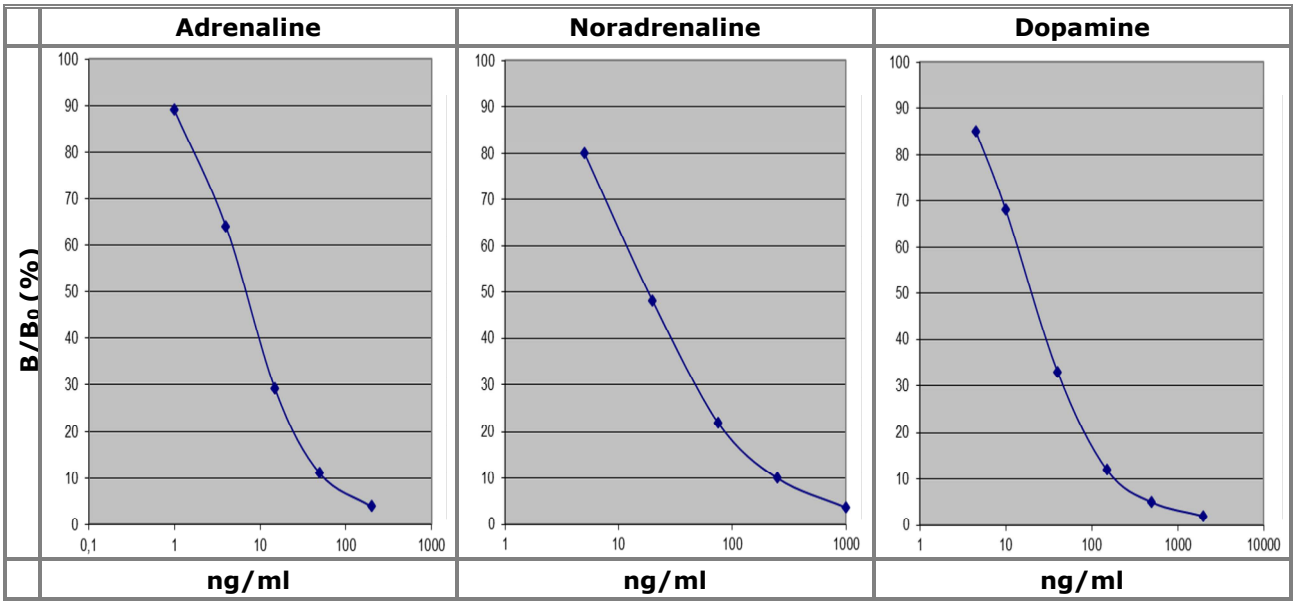
	Adrenaline	Noradrenaline	Dopamine
24-hour urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)	< 600 µg/day (3 900 nmol/day)
Plasma	< 100 pg/ml	< 600 pg/ml	< 100 pg/ml

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 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 standard curves

Examples, do not use for calculation!



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)		Adrenaline	Noradrenaline	Dopamine
	Urine	0.5 ng/ml	1.4 ng/ml	3.8 ng/ml
	Plasma	24 pg/ml	54 pg/ml	39 pg/ml

	Substance	Cross Reactivity (%)		
		Adrenaline	Noradrenaline	Dopamine
Analytical Specificity (Cross Reactivity)	Derivatized Adrenaline	100	0.08	0.02
	Derivatized Noradrenaline	0.13	100	6.4
	Derivatized Dopamine	< 0.01	0.03	100
	Metanephrine	0.18	< 0.01	< 0.01
	Normetanephrine	< 0.01	0.16	0.01
	3-Methoxytyramine	< 0.01	< 0.01	0.49
	3-Methoxy-4-hydroxyphenylglycol	<0.01	< 0.01	< 0.01
	Tyramine	< 0.0007	< 0.01	0.18
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01	< 0.01

Precision

Intra-Assay Urine (n = 40)

	Sample	Range (ng/ml)	CV (%)
Adrenaline	1	15.8 ± 1.1	7.0
	2	22.3 ± 1.7	7.4
	3	45.2 ± 5.1	11.3
Noradrenaline	1	86.8 ± 6.8	7.8
	2	122 ± 10.9	8.9
	3	244 ± 21.9	8.9
Dopamine	1	283 ± 44.3	16.1
	2	338 ± 54.7	15.9
	3	594 ± 170	28.7

Intra-Assay Plasma (n = 40)

	Sample	Range (ng/ml)	CV (%)
Adrenaline	1	146 ± 17.4	12.0
	2	389 ± 38.7	10.1
	3	1 271 ± 88.4	7.0
Noradrenaline	1	992 ± 85.8	9.3
	2	1 811 ± 224	12.3
	3	4 935 ± 663	13.4
Dopamine	1	346 ± 30.8	8.8
	2	880 ± 111	12.3
	3	2 588 ± 742	24.0

Inter-Assay Urine (n = 18)

	Sample	Range (ng/ml)	CV (%)
Adrenaline	1	15.5 ± 1.5	9.5
	2	21.0 ± 2.5	11.7
	3	42.7 ± 5.0	11.7
Noradrenaline	1	77.7 ± 8.5	10.9
	2	104 ± 9.4	9.1
	3	198 ± 22.7	11.5
Dopamine	1	225 ± 34.7	15.4
	2	272 ± 55.8	20.5
	3	452 ± 118	26.0

Inter-Assay Plasma (n = 20)

	Sample	Range (ng/ml)	CV (%)
Adrenaline	1	129 ± 12.7	9.8
	2	347 ± 31.9	9.2
	3	1 139 ± 128	11.3
Noradrenaline	1	754 ± 184	24.4
	2	1 661 ± 176	10.9
	3	4 049 ± 391	9.7
Dopamine	1	260 ± 74.7	28.8
	2	761 ± 173	22.7
	3	2 216 ± 564	25.5

			Serial dilution up to	Range (%)	Mean (%)
Linearity	Adrenaline	Urine	1:128	99 - 114	105
		Plasma	1:128	107 - 113	110
	Noradrenaline	Urine	1:128	93 - 106	98
		Plasma	1:128	95 - 113	103
	Dopamine	Urine	1:128	90 - 121	105
		Plasma	1:128	67 - 90	83

			Concentration range	Range (%)	Mean (%)
Recovery	Adrenaline	Urine	5.2 – 48.8 ng/ml	101 - 111	105
		Plasma	25.3 – 1 001 pg/ml	105 - 119	111
	Noradrenaline	Urine	62.9 – 292 ng/ml	99 - 107	102
		Plasma	377 – 4 457 pg/ml	80 - 101	92
	Dopamine	Urine	137 – 1 823 ng/ml	79 - 126	110
		Plasma	20.3 – 5 158 pg/ml	74 - 94	85

Method Comparison versus HPLC*	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
	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
	Dopamine	Urine	HPLC = 1.07 RIA + 0.01	r = 0.98; n = 21
		Plasma	HPLC = 1.00 RIA + 0.003	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.				

9. References/Literature

(1) Kvetnansky et al. Stress Stimulates Production of Catecholamines in Rat Adipocytes. Cellular and Molecular Neurobiology, 32(5):801-813 (2012)

(2) Wetsch et al. Preoperative stress and anxiety in day-care patients and inpatients undergoing fast-track surgery. British Journal of Anaesthesia, 103 (2):199–205 (2009)

(3) Mahapatra et al. The chromogranin A fragment catestatin: specificity, potency and mechanism to inhibit exocytotic secretion of multiple catecholamine storage vesicle co-transmitters. Journal of Hypertension, 24(5):895-904 (2006)

For orders, please contact:
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⚠ ***For updated literature 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!