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Instructions for use

Dopamine RIA

REF KIPL0300

96 +2 +8 °C IVD CE 200 kBq

Dopamine RIA

1. Intended use and principle of the test

^{125} I – Radioimmunoassay for the quantitative determination of Dopamine in plasma and urine. For in-vitro diagnostic use only.

Dopamine 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.

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 R-0025	PREC-REAG	**Precipitating Reagent**	1 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA R-0320	^{125}I DOP	**^{125}I – Dopamine**	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, green screw cap
BA R-6310	AS DOP	**Dopamine Antiserum**	1 x 5.25 mL	from rabbit, ready for use, green coloured, green screw cap
BA R-6601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-6602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-6603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-6604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-6605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-6606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
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-6613	ASSAY-BUFF	**Assay Buffer**	1 x 6 mL	ready for use, contains 1 M HCl
BA R-6614	COENZYME	**Coenzyme**	1 x 2 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	2 x 1 mL	lyophilized, contains the enzyme COMT
BA R-6617	EXTRACT-BUFF	**Extraction Buffer**	1 x 6 mL	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	ready for use, yellow coloured, contains 0.025 M HCl
BA R-6651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-6652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA R-6609	STANDARD A/B	**Standard A/B**	1 x 4 mL	ready for use

⚠ **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 variable precision micropipettes (e.g. 10-100 µL / 100-1,000µL)
- 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 3mm; approx. 600 rpm)
- Gamma counter
- Vortex mixer
- Absorbent material (paper towel)
- Distilled water

5. Sample collection and storage

Plasma
EDTA-Plasma. 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 500xg to spin down adhering liquids.*

6.1 Preparation of reagents

Acylation Reagent
The Acylation Reagent has a freezing point of 18.5°C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Reagent can be stored at room temperature (20 – 25°C) separate from the other kit components.

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!*

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**, **10 µL** of **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 **distilled water** 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 and 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 distilled water** 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 distilled water** 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 and discard. ⚠ ***Do not decant the supernatant thereafter!*** The following volumes of the supernatant are needed for the subsequent RIA: **Dopamine (standards + urine) 10 µL** **Dopamine (plasma) 25 µL**

6.3 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 minutes** 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 hours (overnight) at 2-8°C. ***Alternatively** incubate for **2 hours 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 minutes** at **2 - 8 °C**.
10.	Centrifuge for **15 minutes** 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 minute** in a gamma counter.

7. Calculation of results

	Concentration of the standards						
Standard	**A**	**B**	**C**	**D**	**E**	**F**	**A/B***
Dopamine (ng/mL)	0	10	40	150	500	2 000	4.5
Dopamine (nmol/L)	0	65	261	980	3 265	13 060	29
Conversion:	Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L)						

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

The calibration curves are obtained by plotting the absorbance readings (calculate the mean absorbance) of 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**

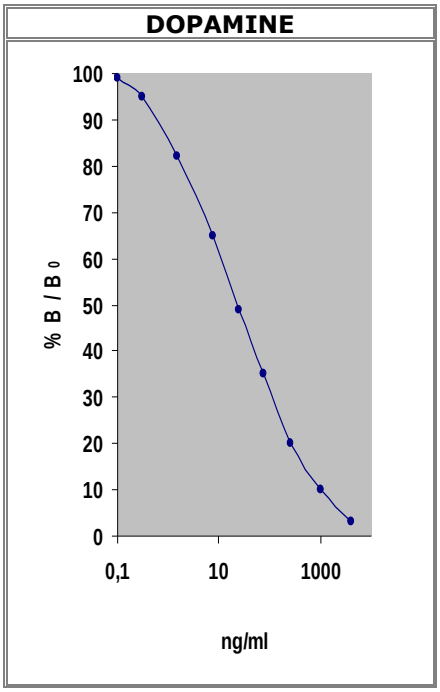
Plasma samples:

The read concentrations of the **plasma samples** have to be **divided by 75.**

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 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



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

Expected Reference Values		**Dopamine**
	Urine	< 600 µg/day (3 900 nmol/day)
	Plasma	< 100 pg/mL

Analytical Sensitivity (Limit of Detection)		**Dopamine**
	Urine	4.5 ng/mL
	Plasma	20 pg/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
		Dopamine
	Derivatized Adrenaline	0.03
	Derivatized Noradrenaline	0.87
	Derivatized Dopamine	100
	Metanephrine	< 0.007
	Normetanephrine	0.008
	3-Methoxytyramine	0.55
	3-Methoxy-4-hydroxyphenylglycol	< 0.007
	Tyramine	0.13
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.007

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Dopamine	1	73 ± 5.9	8.1	Dopamine	1	107 ± 9.7	9.0
	2	260 ± 16	6.1		2	507 ± 68	13.4

Linearity			Range	Serial dilution up to	Range (%)
	Dopamine	Urine	42 -966 ng/mL	1:16	89 – 113
		Plasma	2,000-28,700 pg/mL	1:16	82 – 119

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Dopamine	Urine	109	96 – 127	
		Plasma	79	61 – 100	

Method Comparison versus HPLC*	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.				

⚠ ***Updated literature, information about clinical significance or any other information about the test are available on the homepage or contact the manufacturer directly.***

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!