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Instructions for use

Dopamine Urine RIA Fast Track

REF **BA R-7300**

Σ 96

+2 +8 °C

IVD

CE

200 kBq

Dopamine Urine RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Dopamine in urine

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 ^{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 E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 ml	light purple	concentrated
BA R-0035	PREC-REAG	**Precipitating Reagent**	1 x 22 ml	orange	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	green	ready for use
BA R-0320	^{125}I-DOP	**^{125}I – Dopamine**	1 x 5.5 ml	dark green	ready for use, activity < 200 kBq, red coloured
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-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-7310	AS DOP	**Dopamine Antiserum**	1 x 5.25 ml	dark green	ready for use, from rabbit, green coloured
BA R-7601	STANDARD A	**Standard A**	1 x 4 ml	white	ready for use
BA R-7602	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA R-7603	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA R-7604	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA R-7605	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA R-7606	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
BA R-7617	TE-BUFF	**TE Buffer**	1 x 6 ml	brown	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	brown	ready for use, yellow coloured, contains 0.025 M HCl
BA R-7651	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA R-7652	CONTROL 2	**Control 2**	1 x 4 ml	dark red	ready for use

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

- Calibrated precision pipettes to dispense volumes between 25 – 200 µl
- Conical tubes (e.g. SARSTEDT RIA tubes) and suitable rack
- Suitable device for aspirating or decanting
- Plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Centrifuge capable of at least 3 000 x g
- Gamma counter
- Vortex mixer
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)

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 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!*

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) 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**, **controls** and **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. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** 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. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** 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 volume of the supernatant is needed for the subsequent RIA: **Dopamine 25 µl**

6.3 Dopamine RIA

1.	Pipette **25 µl** of **Release Buffer** into the **tubes** for the **NSB**.
2.	Pipette **25 µl** of the **derivatized standards, controls,** and **samples** into the respective **tubes**.
3.	Pipette **50 µl** of the **^{125}I Dopamine** into **all tubes**.
4.	Pipette **50 µl** of **Dopamine-Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly.
5.	Cover tubes. Incubate for **60 min** 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 min** at **2 - 8 °C**.
8.	Centrifuge for **15 min** 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 min** in a gamma counter.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Dopamine (ng/ml)	0	25	75	250	1 000	4 000
Dopamine (nmol/l)	0	163	490	1 633	6 530	26 120
Conversion:	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).

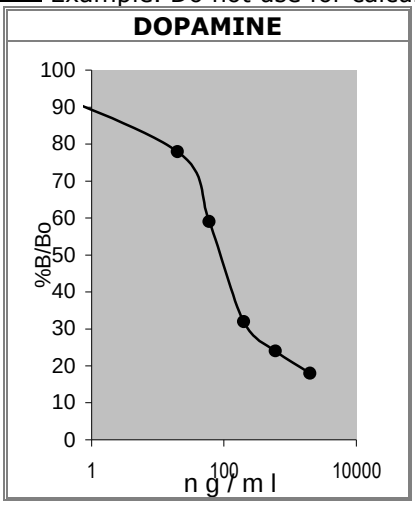
Samples and controls:
The concentrations of the **samples** and the **controls** can be read directly from the standard curve.

7.1 Quality control

It is recommended to use control samples according to national 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

⚠ Example. Do not use for calculation!



8. Assay characteristics

Expected Reference Values		Dopamine
	Urine	< 600 µg/day (3,900 nmol/day)

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD	
		Dopamine
	Urine	3 ng/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, Homovanillic acid, Tyrosine, L-Dopa, 3-Methoxy-4-hydroxymandelic acid	< 0.007

Precision							
Intra-Assay				**Inter-Assay**			
		Range (ng/ml)	CV (%)			Range (ng/ml)	CV (%)
Dopamine	Urine	72.7 - 741	7.9	Dopamine	Urine	69.9 - 634	8.4

Linearity			Range (ng/ml)	Serial dilution up to	Range (%)
	Dopamine	Urine	43 - 688	1:16	89 - 100

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Dopamine	Urine	93	84 - 100	

Method Comparison versus HPLC	Dopamine	Urine	HPLC = 0.7387 x RIA +0.370	r^2 = 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	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!