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LDN[®]

Instructions for use
2-MET Urine RIA Fast Track

REF

BA R-8600



IVD



400 kBq

1. Intended use and principle of the test

¹²⁵I – Radioimmunoassay for the quantitative determination of Metanephrine and Normetanephrine in urine.

First, Metanephrine (Metadrenaline) and Normetanephrine (Normetadrenaline) are quantitatively acylated.

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 ¹²⁵Iodine (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.

4.1 **Contents of the kit**

BA D-0023	REAC-TUBES	Reaction Tubes	2 x 50	ready for use
BA R-0012	ACYL-CONC	Acylation Concentrate	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
BA R-0025	PREC-REAG	Precipitating Reagent	2 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. <i>Mix thoroughly before use!</i>
BA R-0075	ACYL-DILUENT	Acylation Diluent	1x 4 mL	ready for use
BA R-0120	¹²⁵ I ADR MN	125I - Adrenaline - Metanephrine	1 x 5.5 mL	activity 2x < 100 kBq, ready for use, red coloured, blue screw cap
BA R-0220	¹²⁵ I NAD NMN	125I - Noradrenaline - Normetanephrine	1 x 5.5 mL	activity 2x < 100 kBq, ready for use, red coloured, yellow screw cap
BA R-8410	AS MN	Metanephrine Antiserum	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-8510	AS NMN	Normetanephrine Antiserum	1 x 5.25 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA R-8601	STANDARD A	Standard A	1 x 4 mL	ready for use
BA R-8602	STANDARD B	Standard B	1 x 4 mL	ready for use
BA R-8603	STANDARD C	Standard C	1 x 4 mL	ready for use
BA R-8604	STANDARD D	Standard D	1 x 4 mL	ready for use
BA R-8605	STANDARD E	Standard E	1 x 4 mL	ready for use
BA R-8606	STANDARD F	Standard F	1 x 4 mL	ready for use
BA R-8611	ACYL-BUFF	Acylation Buffer	1 x 30 mL	ready for use
BA R-8619	HCL	Hydrochloric Acid	1 x 30 mL	ready for use, contains 0.25 M HCl, yellow coloured, green screw cap
BA R-8651	CONTROL 1	Control 1	1 x 4 mL	ready for use
BA R-8652	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. 10-100 µL / 100-1000 µL)
- Polystyrene tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- Temperature controlled water bath (37°C, 90°C) or similar heating device
- Gamma counter, -Vortex mixer, -Distilled water

5. **Sample collection and storage**

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.

Determine the total volume of urine excreted during 24 h for calculation of the results!

Storage: for longer period (up to 6 months) at -20°C.

Repeated freezing and thawing of the samples 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 500xg to spin down adhering liquids.

6.1 Preparation of reagents

Acylation Solution

⚠ Before preparing the Acylation Solution make sure that the Acylation Diluent (BA R-0075) has reached room temperature ($\geq 20^{\circ}\text{C}$) and forms a homogenous, crystal-free solution.

Dilute the Acylation Concentrate (BA R-0012) 1 + 60 with Acylation-Diluent in a glass or polypropylene vial.

Acylation Concentrate	10 μL	20 μL	25 μL	50 μL
Acylation-Diluent	600 μL	1.2 mL	1.5 mL	3 mL

⚠ The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!

6.2 Preparation and acylation

Hydrolysis

1. Pipette 25 μL of standards , 25 μL of controls , and 25 μL of urine samples into the respective Reaction Tubes .
2. Add 250 μL Hydrochloric Acid to all tubes.
3. Mix thoroughly (vortex) and hydrolyze for 30 min. at 90 $^{\circ}\text{C}$.
4. Let the tubes cool down to room temperature.
⚠ For the measurement of the free metanephrine and free normetanephrine only, leave away steps 3 and 4.

Acylation

1. Pipette 250 μL of Acylation Buffer into all tubes.				
2. Add 25 μL of Acylation Solution to all tubes.				
3. Mix thoroughly (vortex) and acylate for 15 minutes at RT (20-25$^{\circ}\text{C}$) .				
4. Add 1 mL dist. water to all tubes.				
⚠ The following volumes of the eluate are needed for the subsequent RIA: <table border="1" data-bbox="199 1388 1053 1433"><tr><td>Metanephrine</td><td>25 μL</td><td>Normetanephrine</td><td>25 μL</td></tr></table>	Metanephrine	25 μL	Normetanephrine	25 μL
Metanephrine	25 μL	Normetanephrine	25 μL	

6.3 Metanephrine RIA

1. Pipette 25 μL of the acylated Standard A into polystyrene tubes for the NSB .
2. Pipette 25 μL of the acylated standards, controls and samples into the respective polystyrene tubes.
3. Pipette 50 μL of the ^{125}I Metanephrine into all tubes .
4. Pipette 50 μL of Metanephrine Antiserum into all tubes (except totals and NSB) ; mix thoroughly.
5. Cover tubes. Incubate for 60 minutes at 37$^{\circ}\text{C}$.
6. Mix the chilled (2 - 8 $^{\circ}\text{C}$) Precipitating Reagent thoroughly, pipette each 500 μL into all tubes (except totals) , and mix on a vortex.
7. Incubate for 15 minutes at 2 - 8 $^{\circ}\text{C}$.
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.

6.4 Normetanephrine RIA

1.	Pipette 25 µL of the acylated Standard A into the polystyrene tubes for the NSB .
2.	Pipette 25 µL of the acylated standards, controls and samples into the respective polystyrene tubes.
3.	Pipette 50 µL of the ¹²⁵ I Normetanephrine into all tubes .
4.	Pipette 50 µL of Normetanephrine Antiserum into all tubes (except totals and NSB) ; mix thoroughly.
5.	Cover tubes. Incubate for 60 minutes at 37°C .
6.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 500 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

Standard	Concentration of the standards					
	A	B	C	D	E	F
Normetanephrine (ng/mL=µg/L)	0	30	90	300	900	3 000
Normetanephrine (nmol/L)	0	164	491	1 638	4 914	16 380
Metanephrine (ng/mL=µg/L)	0	20	60	200	600	2 000
Metanephrine (nmol/L)	0	101	304	1 014	3 042	10 140
Conversion:	Normetanephrine (ng/mL) x 5.46 = Normetanephrine (nmol/L) Metanephrine (ng/mL) x 5.07 = Metanephrine (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 read off, is obtained by plotting the percentage of (B-NSB)/(B0-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).

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

The amount of analyte excreted per day (µg/day) is calculated according to:

$$\text{concentration of the sample (in } \mu\text{g/L)} \times \text{volume of urine excreted per day (in L/day)}$$

Example

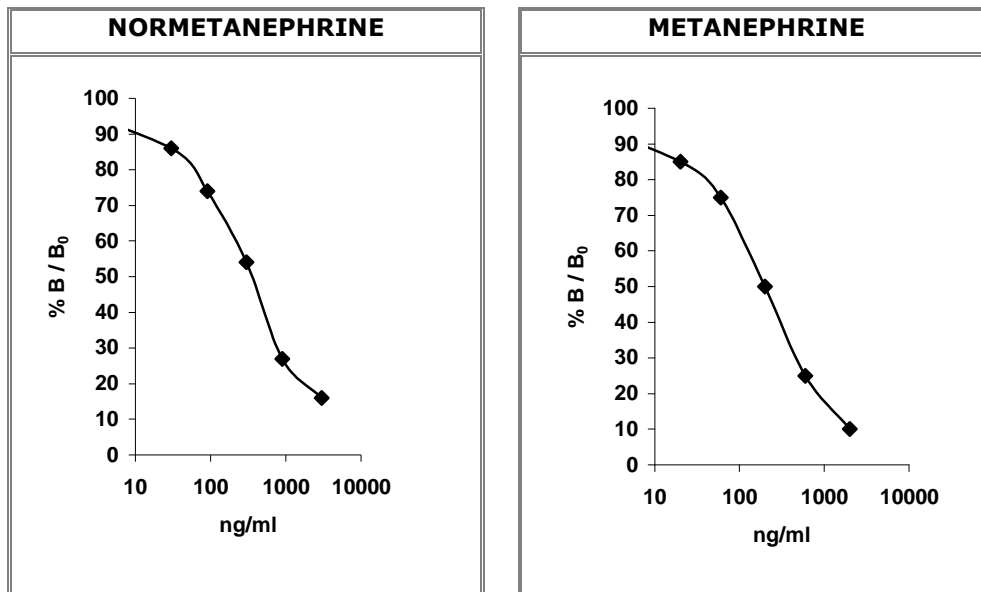
The concentration of the sample read from the curve is 125 µg/L. The amount of urine collected during 24 hours is 1.3 L. Then the amount of analyte excreted during one day would be:

$$125 \mu\text{g/L} \times 1.3 \text{ L/day} = 162.5 \mu\text{g/day}$$

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 indicated on the QC-report.

7.2 Typical calibration curves (Examples. Do not use for calculation!)



8. Assay characteristics

Expected Reference Values	Metanephrine		Normetanephrine	
	Urine	< 350 µg/day	< 600 µg/day	

Analytical Sensitivity (Limit of Detection)	Metanephrine		Normetanephrine	
	Urine	13 ng/mL	11 ng/mL	

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
	Derivatized Metanephrine	100	0.06
	Derivatized Normetanephrine	0.1	100
	Derivatized 3-methoxytyramine	0.002	0.08
	Adrenaline	1.73	< 0.001
	Noradrenaline	< 0.001	1.07
	Dopamine	< 0.001	0.001
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	< 0.001	< 0.001


Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Metanephrine	1	75 ± 7.6	10.1	Metanephrine	1	57 ± 4.0	7.6
	2	350 ± 17	4.8		2	398 ± 19	4.9
Normetanephrine	1	189 ± 12	6.6	Normetanephrine	1	230 ± 12	5.2
	2	1,095 ± 73	6.6		2	1,596 ± 100	6.3

Linearity			Range	Serial dilution up to	Mean (%)
	Metanephrine	Urine	42 – 803 ng/mL	1:16	109
	Normetanephrine	Urine	105 – 2,528 ng/mL	1:16	90







Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Metanephrine	Urine	94	86 – 102	
	Normetanephrine	Urine	100	86 – 111	

Method comparison versus HPLC*			r = 0.99; n = 21	
	Metanephrine	Urine	HPLC = 1.02 RIA - 0.3	
Normetanephrine	Urine	HPLC = 1.1 RIA - 0.3		

*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. 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.

 **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!