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Instructions for use

Metanephrine Plasma RIA Fast Track

REF KIPL0700

100 +2 +8 °C IVD CE 200 kBq

Metanephrine Plasma RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of free Metanephrine in plasma.

First, the plasma proteins are removed by precipitation. Then the metanephrine (metadrenaline) is 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.

⚠ The antisera used in this test kit only recognise the biologically relevant L-forms of metanephrines. Commercially available synthetic normetanephrine or metanephrine is always a mixture of the D- and L-forms. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic metanephrines are used to enrich native samples. As only about 50% of the synthetic metanephrines, i.e. the L-portion, will be detected by use of this kit, these samples will be underestimated. Therefore only native samples should be used.

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

Store the reagents at 2 - 8 °C until expiry date. Do not use components beyond the expiry 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 D-0023	REAC-TUBES	**Reaction Tubes**	2 x 50 tubes	ready for use
BA R-0030	PREC-REAG	**Precipitating Reagent**	1 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer *Mix thoroughly before use!*
BA R-0028	EQUA-REAG	**Equalizing Reagent**	2 x 10 mL	lyophilized
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	2 x 4 mL	ready for use
BA R-0120	^{125}I ADR MN	**^{125}I – Adrenaline - Metanephrine**	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, blue screw cap
BA R-7110	AS ADR MN	**Adrenaline - Metanephrine Antiserum**	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-8301	STANDARD A	**Standard A**	1 x 12 mL	ready for use
BA R-8302	STANDARD B	**Standard B**	1 x 2 mL	ready for use
BA R-8303	STANDARD C	**Standard C**	1 x 2 mL	ready for use
BA R-8304	STANDARD D	**Standard D**	1 x 2 mL	ready for use
BA R-8305	STANDARD E	**Standard E**	1 x 2 mL	ready for use
BA R-8306	STANDARD F	**Standard F**	1 x 2 mL	ready for use
BA R-8312	ACYL-CONC	**Acylation Concentrate**	1 x 1.5mL	concentrated
BA R-8351	CONTROL 1	**Control 1**	1 x 2 mL	ready for use
BA R-8352	CONTROL 2	**Control 2**	1 x 2 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 / 100-1,000µL)
- Conical tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- for the alternative protocol with short incubation times, a shaker is needed (amplitude 3 mm; approx. 600 rpm)
- Gamma Counter
- Vortex mixer
- Distilled water

5. Sample collection and storage

EDTA- or citrate-plasma has to be used. Haemolytic and especially lipemic samples should not be used in the assay.
Storage: up to 6 hours at 2 - 8°C, for longer periods (up to 6 months) at - 20°C.
Repeated freezing and thawing should be avoided.

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

Equalizing Reagent
The Equalizing Reagent has to be reconstituted with 10 mL distilled water.
Reconstituted Equalizing Reagent which is not used immediately has to be frozen at -20 °C (in aliquotes) and may be thawed only once.

Acylation Solution
Pipette 80 µL Acylation Reagent Concentrate to 3 mL distilled water and mix thoroughly.
Use immediately!

⚠ *The Acylation Solution is stable for only 3 minutes.*

6.2 Precipitation

1.	Pipette **100 µL** of **standards**, **100 µL** of **controls,** and **500 µL** of **plasma samples** into the respective **Reaction Tubes.**
2.	Add **500 µL Equalizing Reagent** to all tubes containing **standards** and **controls**.
3.	Add **100 µL Standard A** to all tubes containing **plasma samples**.
4.	Mix the **Reaction Tubes** thoroughly (vortex) and centrifuge for **15 minutes** at **3 000 x g**.
⚠	Take **100 µL** of the clear supernatant for the Metanephrine RIA

6.3 Metanephrine RIA

⚠ *The use of conical tubes for the RIA is highly recommended!*

1.	Pipette **100 µL** of **distilled water** into the tubes for the **NSB**.
2.	Pipette **100 µL** of the **clear supernatants** of **standards, controls** and **samples** into the respective tubes.
3.	Pipette **50 µL** of **Adjustment Buffer** into all tubes (***except totals***).
4.	Pipette **25 µL Acylation Solution** (refer to 6.1*)* into all tubes (***except totals***).
⚠	***The Acylation Solution is stable for only 3 minutes.***
5.	Mix thoroughly (vortex) and incubate for **15 minutes** at **RT** (20-25°C).
6.	Pipette **50 µL** of **Metanephrine Antiserum** into **all tubes** (***except totals and NSB***); mix thoroughly (vortex).
7.	Incubate for **1 hour** at **RT** (20-25°C).
8.	Pipette **50 µL** of the **^{125}I Metanephrine** into **all tubes** and mix thoroughly (vortex).
9.	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).*
10.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **0.5 mL** into **all tubes** (***except totals***), and mix on a vortex.
11.	Incubate for **15 minutes** at **2 - 8 °C**.
12.	Centrifuge for **15 minutes** at **3 000 x g**, if possible in a refrigerated centrifuge.
⚠	***Continue without any delay with step 13.***
13.	**Decant** or aspirate the **supernatant** **carefully** (***except totals***). Blot the tubes dry and leave them upside for 2 minutes.
14.	**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**
Metanephrine (pg/mL)	0	36	120	360	1 200	3 600
Metanephrine (pmol/L)	0	183	608	1 830	6 080	18 300
Conversion:	Metanephrine (pg/mL) x 5.07 = Metanephrine (pmol/L)					

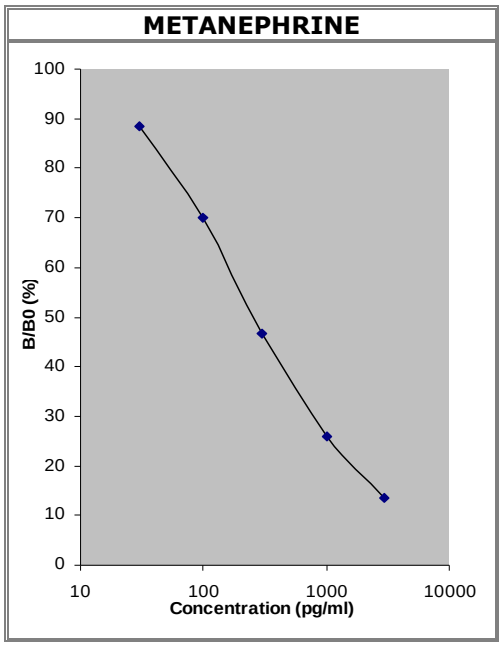
Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The calibration curve, from which the concentrations in the samples can be taken, is obtained by using the percentage of (B-NSB)/(B0-NSB) measured for the standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations of the **samples** and **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, 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 Reference Values		Metanephrine
	Plasma	< 90 pg/mL

Analytical Sensitivity (Limit of Detection)		Metanephrine
	Plasma	19 pg/mL

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Metanephrine
	Derivatized Metanephrine	100
	Derivatized Normetanephrine	0.04
	3-Methoxytyramin.HCl	< 0.001
	Adrenaline	< 0.001
	Noradrenaline	< 0.001
	Dopamin.HCl	< 0.001
	VMS	< 0.001
	HMVS	< 0.001
	L-DOPA	< 0.001
	L-Tyrosin	< 0.001
	Tyramine.HCl	< 0.001
	Normetanephrine	< 0.001
	Acetaminophen	< 0.001

Intra-Assay Precision				Inter-Assay Precision			
	Sample	Range (pg/mL)	CV (%)		Sample	Range (pg/mL)	CV (%)
Metanephrine	1	185 ± 18	9.8	Metanephrine	1	217 ± 30	14
	2	372 ± 32	8.7		2	388 ± 47	12
	3	891 ± 131	15		3	781 ± 87	11

Linearity			Range	Serial dilution up to	Range (%)
	Metanephrine	Plasma	25 - 2100	1: 65	91

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Metanephrine	Plasma	103	85 - 122	

Method Comparison RIA vs. LC-MS/MS	Metanephrine	Plasma	LC-MS/MS = x – 13.2	r = 0.99; n = 50

⚠ ***For actual 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	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!