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Instructions for use

3-Methoxytyramin Plasma RIA Fast Track

¹²⁵I – Radioimmunoassay for the determination of free 3-Methoxytyramine (3-MT) in plasma.

For research use only, not for use in diagnostic procedures.

REF

IB88528



200 kBq

3-Methoxytyramine Plasma RIA Fast Track**1. Introduction****1.1 Intended use**

¹²⁵I – Radioimmunoassay for the determination of free 3-Methoxytyramine (3-MT) in plasma. For research use only, not for use in diagnostic procedures.

1.2 Background/Principle of the Test**Related Products:**

2-MET Plasma ELISA <small>Fast Track</small>	2-MET Plasma RIA <small>Fast Track</small>
Metanephrine Plasma ELISA <small>Fast Track</small>	Metanephrine Plasma RIA <small>Fast Track</small>
Normetanephrine Plasma ELISA <small>Fast Track</small>	Normetanephrine Plasma RIA <small>Fast Track</small>

3-Methoxytyramine is first extracted using an ion exchange matrix followed by an acylation process. The assay procedure uses 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.

Metanephrine, Normetanephrine and 3-Methoxytyramine are the metabolites of the catecholamines Epinephrine, Norepinephrine and Dopamine, respectively. Cells derived from neuroendocrine tumors (e.g. pheochromocytoma) are known to produce catecholamines, which are secreted episodically via vesicles into the blood stream. But beside this, a small portion of the catecholamines is metabolized inside the cells to the corresponding catecholamines metabolites – namely Metanephrine, Normetanephrine and 3-Methoxytyramine – which are secreted at low levels continuously into the blood stream.

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 a certain type 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) Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- (4) The principles of Good Laboratory Practice (GLP) have to be followed.
- (5) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (6) All kit reagents and samples – with the exception of Precipitating Reagent - should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
- (7) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (8) The radioactive material (¹²⁵Iodine, 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. Products are dispatched on the basis of the nuclear and radiation protection regulations.
- (9) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (10) 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.
- (11) Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
- (12) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (13) A standard curve must be established for each run.
- (14) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.

- (15) 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.
- (16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
- (17) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (18) Kit reagents must be regarded as hazardous waste and disposed 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 hemolytic or lipemic might cause inaccurate results.

2.2.2 Drug interferences

Please refer to point "Sample collection and storage".

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-0030	PREC-REAG	Precipitating Reagent - Ready to use
Content:	Goat anti-rabbit serum in PEG phosphate buffer	
Volume:	1 x 55 ml/vial, yellow cap	
BA R-0320	¹²⁵I DOP	¹²⁵I - Dopamine / 3-Methoxytyramine - Ready to use
Content:	¹²⁵ I labeled 3-Methoxytyramine, red coloured	
Volume:	1 x 5.5 ml/vial, dark green cap	
Hazards identification:		Radioactive, activity < 200 kBq
BA R-6310	AS DOP	Dopamine/3-Methoxytyramine Antiserum - Ready to use
Content:	Rabbit anti- 3-Methoxytyramine antibody, green coloured	
Volume:	1 x 5.25 ml/vial, dark green cap	
BA E-2442	EXTRACT-PLATE 48	Extraction Plate - Ready to use
Content:	2 x 48 well plate, precoated with cation exchanger in a resealable pouch	
BA R-8325	CLEAN-CONC 25x	Cleaning Concentrate - 25x concentrated
Content:	Buffer with sodium acetate	
Volume:	1 x 20 ml/vial, brown cap	
BA R-8326	ELUTION-BUFF	Elution Buffer - Ready to use
Content:	0.1 M sodium hydroxide, dark purple coloured	
Volume:	1 x 14 ml/vial, dark green cap	

BA R-8327 **ADJUST-BUFF** **Adjustment Buffer** - Ready to use

Content: TRIS buffer with BSA and a non-mercury stabilizer

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

Standards and Controls - Ready to use

Cat. no.	Component	Colour/Cap	Concentration	Concentration	Volume/Vial
			pg/ml 3-MT	pmol/l 3-MT	
BA R-8801	STANDARD A	white	0	0	6 ml
BA R-8802	STANDARD B	light yellow	2	12	4 ml
BA R-8803	STANDARD C	orange	5	30	4 ml
BA R-8804	STANDARD D	dark blue	15	90	4 ml
BA R-8805	STANDARD E	light grey	50	299	4 ml
BA R-8806	STANDARD F	black	150	897	4 ml
BA R-8851	CONTROL 1	light green	Refer to QC report for expected value and acceptable range!		4 ml
BA R-8852	CONTROL 2	dark red			4 ml

Conversion: 3-Methoxytyramine (pg/ml) x 5.98 = 3-Methoxytyramine (pmol/l)

Content: Acidic buffer with non-mercury stabilizer, spiked with a defined quantity of 3-Methoxytyramine

BA R-8811 **ACYL-BUFF** **Acylation-Buffer** - Ready to use

Content: 0.24 M HCl

Volume: 1 x 3ml/vial, brown cap

BA R-8823 **ACYL-TUBES** **Acylation-Tubes** - Ready to use

Content: PS tubes coated with acylation reagent in a resealable pouch

Volume: 4 x 25 tubes

BA R-8828 **EQUA-REAG** **Equalizing-Reagent** - Ready to use

Content: Human serum, negative for HIV I/II, HBsAg and HCV

Volume: 3 x 14 ml, white cap

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

- Calibrated precision pipettes to dispense volumes between 25 – 1000 µl
- RIA tubes (Polystyrene) for Total counts (T)
- Suitable rack for RIA tubes
- Centrifuge (preferable refrigerated) capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Gamma Counter
- Vortex mixer
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage


Medications like Serotonin-Noradrenaline reuptake inhibitors, tricyclic antidepressants, MAO inhibitors, antihypertensive drugs and L-DOPA can influence 3-Methoxytyramine level. Sympathomimetic agents, sport and smoking can influence 3-Methoxytyramine level. Alcohol and caffeinated drinks should be avoided the day before and including the day of sample collection. Consumption of food products that contain substantial quantities of biogenic amines (e.g. chocolate, some fruits (e.g. banana, pineapple), fruit drinks, nuts, tomatoes) can cause significant increase in plasma free 3-Methoxytyramine level. Dietary restrictions are necessary prior to sampling.

EDTA- or Heparin-Plasma

Whole blood should be collected into centrifuge tubes (Monovette™ or Vacuette™) containing EDTA or heparin as anti-coagulant and centrifuged (according to manufacturer's instructions) immediately after collection. Haemolytic and lipemic samples should not be used for the assay. Storage: up to 6 hours at 2 - 8 °C, for longer period (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 extraction wells/acylation 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

Cleaning Buffer


Dilute the 20 ml Cleaning Concentrate (BA R-8325) with water (deionized, distilled, or ultra-pure) to a final volume of 500 ml.

Storage: 1 month 2 - 8 °C

6.2 Preparation of samples

 Due to a cross reaction of the 3-Methoxytyramine antiserum to Normetanephrine, the Normetanephrine plasma concentrations should be determined parallel to this assay. Please refer to point 7 for the correction of the 3-Methoxytyramine result.

6.3 Precipitation

1.	Pipette 50 µl of standards and controls into the respective wells of the Extraction Plate .
2.	Add 50 µl Standard A to all wells for plasma samples .
3.	Add 750 µl of Equalizing Reagent to the wells with standards and controls .
4.	Pipette 750 µl of plasma samples to the respective wells.
5.	Incubate plate 2 h at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
6.	Empty plate and blot dry by tapping the inverted plate on absorbent material.
7.	Wash the plate 3 x by adding 1 ml of Cleaning Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
8.	Pipette 100 µl of Elution Buffer into all wells.
9.	Cover plate with adhesive foil. Incubate 15 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm). Remove the foil.
	Do not decant the supernatant thereafter! 90 µl of the supernatant are needed for the subsequent RIA (Step 3 next Chapter)

6.4 3-Methoxytyramine RIA

⚠ *Acylation tubes are used for the RIA!*

For determination of the total counts you can use usual Polystyrene RIA tubes.

1.	Pipette 25 µl of Acylation Buffer into all Acylation Tubes (except totals) . The Acylation Tubes can be used only once!
Do not change pipetting order at this stage (always Acylation Buffer first)!	
2.	Pipette 90 µl of Elution Buffer into the Acylation Tubes for the NSB .
3.	Pipette 90 µl of the extracted standards, controls and samples into the respective Acylation Tubes .
⚠	<i>Due to the low elution volume it is advisable to hold the Extraction Plate tilted (~ 45°) while the standards, controls and samples are pipetted into the tubes.</i>
4.	Pipette 25 µl of Adjustment Buffer into all tubes (except totals) .
5.	Mix thoroughly (vortex) and incubate for 15 min at RT (20 - 25 °C).
6.	Pipette 50 µl of Dopamine/3-Methoxytyramine Antiserum into all tubes (except NSB) ; mix thoroughly (vortex).
7.	Cover tubes. Incubate for 1 h at RT (20 - 25 °C).
8.	Pipette 50 µl of the ¹²⁵I Dopamine/3-Methoxytyramine into all tubes and mix thoroughly (vortex). Pipette also 50 µl of the ¹²⁵I Dopamine/3-Methoxytyramine into additional empty polystyrene tubes for determination of the total counts .
9.	Cover tubes. Incubate for 15 - 20 h (overnight) at 2 - 8 °C .
⚠	Avoid a temperature gradient within the rack during incubation!
10.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 500 µl into all tubes (except totals) , and mix on a vortex.
11.	Incubate for 15 min at 2 - 8 °C .
12.	Centrifuge for 15 min 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 (on absorbent material) and leave them upside for 2 minutes.
14.	Count all tubes for 1 min in a gamma counter.

7. Calculation of results

Measuring range	3-Methoxytyramine
	1.95 - 150 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 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).

⚠ *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.*

Controls:

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

⚠ Plasma samples:

The concentrations read from the standard curve have to be corrected for Normetanephrine cross reactivity:

<p>Effective 3-MT-concentration =</p> <p>read [3-MT pg/ml] - 0.016 x [Normetanephrine pg/ml]</p>

Example

The 3-Methoxytyramine concentration taken from the standard curve is 10 pg/ml. The determined Normetanephrine concentration from the same sample is 350 pg/ml.
Effective 3-MT-concentration:

$$10 \text{ pg/ml} - 0.016 \times 350 \text{ pg/ml} = 4.4 \text{ pg/ml}$$

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Equalizing Reagent (BA R-8828) and have to be re-assayed.

Conversion

$$3\text{-Methoxytyramine (pg/ml)} \times 5.98 = 3\text{-Methoxytyramine (pmol/l)}$$

Expected reference values

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


In a study conducted with EDTA Plasma of apparently normal healthy donors (n = 125, blood samples taken in sitting position), using the 3-Methoxytyramine Plasma RIA ^{Fast Track} the following value was observed.

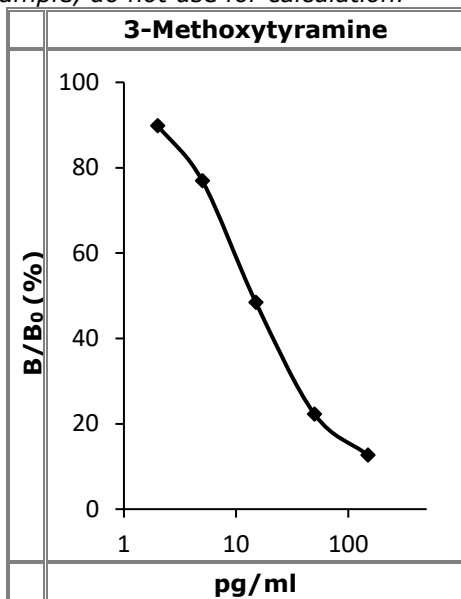
3-Methoxytyramine
< 8 pg/ml

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, 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 standard curve

 Example, do not use for calculation!



8. Assay characteristics

Analytical Sensitivity		3-Methoxytyramine
	LOD (pg/ml)	1.10
	LOQ (pg/ml)	1.95

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
	Normetanephrine	1.34
	Metanephrine	<0.01
	Adrenaline	<0.01
	Noradrenaline	<0.01
	Dopamine	<0.01
	Vanillic mandelic acid	<0.01
	Homovanillic acid	<0.01
	L-DOPA	<0.01
	L-Tyrosin	<0.01
	Tyramine	0.04

Precision					
Intra-Assay			Inter-Assay		
Sample	Mean (pg/ml)	CV (%)	Sample	Mean (pg/ml)	CV (%)
1	5.9	13.5	1	4.9	13.0
2	8.6	11.4	2	7.4	12.2
3	17.4	10.9	3	15.3	13.6
4	30.6	12.8	4	27.7	13.1

Linearity	Serial dilution up to	Mean (%)	Range (%)
	1:64	92	81 - 96

Recovery	Mean (%)	Range (%)
	95	90 - 100







Method Comparison RIA vs LC-MS/MS ⁽¹⁾	$y=0.88x + 0.081; r^2=0.99; n=49$

9. References/Literature

- (1) De Jong et al. Plasma free metanephrine measurement using automated online solid phase extraction HPLC-Tandem mass spectrometry. Clin Chem, 53(9): 1684-1693 (2007)
- (2) Eisenhofer et al. Laboratory evaluation of pheochromocytoma and paraganglioma. Clin Chem, 60:1486-1499 (2014)
- (3) Eisenhofer et al. Plasma metadrenalines: Do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin Sci (Lond),88:533-542 (1995)
- (4) Berkel et al. Diagnosis of endocrine disease: Biochemical diagnosis of pheochromocytoma and paraganglioma. Eur J Endocrinol, 170: R109-R119
- (5) Manz et al. Development of enantioselective immunoassays for free plasma metanephrines. Ann.N.Y.Acad.Sci., 1018:582-587 (2004)
- (6) De Jong et al. Dietary Influences on Plasma and Urinary Metanephrines: Implications for Diagnosis of Catecholamine-Producing Tumors. J Clin Endocrinol Metab, 94(8):2841-2849 (2009)
- (7) Deutschbein et al. Influences of Various Confounding Variable and Storage Conditions on Metanephrine and Normetanephrine Levels in Plasma. Clin Endocrinol, 72(2):153-160 (2010)

 **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	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!