Manufactured for Immuno-Biological Laboratories Inc.
(IBL-America)
8201 Central Avenue, NE, Suite P
Minneapolis, MN 55432
Tel: 763-780-2955
Toll Free: 1-888-523-1246

Instructions for use

3-Methoxytyramine Plasma RIA Fast Track

REF **BA R-8800**

2°C–8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

200 kBq

English

3-Methoxytyramine Plasma RIA Fast Track

1. Introduction

1.1 Intended use and principle of the test

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

Related Products:

2-MET Plasma ELISA Fast Track	2-MET Plasma RIA Fast Track
Metanephrine Plasma ELISA Fast Track	Metanephrine Plasma RIA Fast Track
Normetanephrine Plasma ELISA Fast Track	Normetanephrine Plasma RIA Fast Track

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 ^{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.

1.2 Background

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 specimens – 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 specimens.
(7) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(8) 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. 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) 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 haemolytic 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 ^{125}I DOP **^{125}I – Dopamine / 3-Methoxyramine** - 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 pg/ml 3-MT	Concentration pmol/l 3-MT	Volume/Vial
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 – 1 000 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, tryicyclic antidepressants, MAO inhibitors, antihypertensive drugs and L-DOPA can influence 3-Methoxytyramine level. People who are taking such medication should consult with their doctor before specimen collection.
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 gently 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-Methoxythyramine 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 Extraction

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**. *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.*
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 totals and NSB)***; mix thoroughly (vortex).
7.	Cover tubes. Incubate for **1 h** at **RT** (20 - 25 °C).
8.	Pipette **50 µl** of the **^{125}I Dopamine/3-Methoxytyramine** into **all tubes** and mix thoroughly (vortex). Pipette also **50 µl of the ^{125}I 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)/ (B_0-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:

Effective 3-MT-concentration =

read [3-MT pg/ml] - 0.008 x [Normetanephrine pg/ml]

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 pg/ml – 0.008 x 350 pg/ml= 4.4 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-Methoxytyramine (pg/ml) x 5.98 = 3-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.

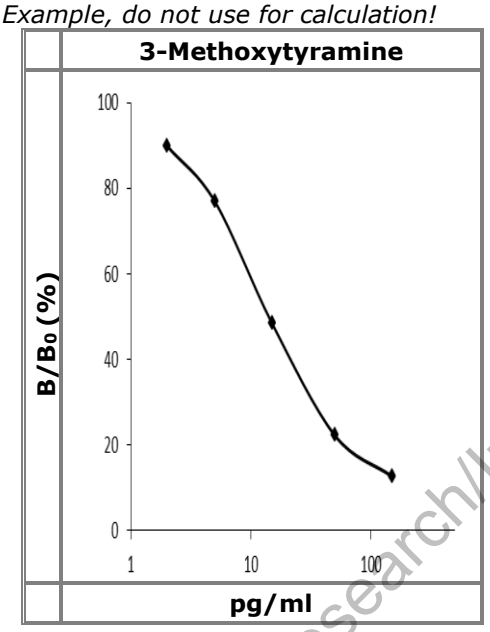
3-Methoxytyramine
< 8 pg/ml

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

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

	Substance	Cross Reactivity (%)
Analytical Specificity (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

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

	Mean (%)	Range (%)
Recovery	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 phaeochromocytoma 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		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!