LDN Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

Instructions for use

2-MET Plasma RIA Fast Track

REF **BA R-8300**

Σ 2 x 100

+2 +8 °C

IVD

CE

400 kBq

Metanephrine–Normetanephrine Plasma RIA

1. Intended use and principle of the test

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

First, the plasma proteins are removed by precipitation. Then the 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 ^{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.

⚠ 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. 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 expiry 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 D-0023	REAC-TUBES	**Reaction Tubes**	2 x 50 tubes		ready for use
BA R-0030	PREC-REAG	**Precipitating Reagent**	2 x 55 ml	yellow	ready for use, goat anti-rabbit serum in PEG phosphate buffer *Mix thoroughly before use!*
BA R-0028	EQUA-REAG	**Equalizing Reagent**	2 x	dark green	lyophilized
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	3 x 4 ml	green	ready for use
BA R-0120	^{125}I ADR MN	**^{125}I – Adrenaline - Metanephrine**	1 x 5.5 ml	blue	ready for use, activity < 200 kBq, red coloured
BA R-0220	^{125}I NAD NMN	**^{125}I – Noradrenaline - Normetanephrine**	1 x 5.5 ml	yellow	ready for use, activity < 200 kBq, red coloured
BA R-7110	AS ADR MN	**Adrenaline - Metanephrine Antiserum**	1 x 5.25 ml	blue	ready for use, from rabbit, blue coloured
BA R-8210	AS NMN	**Normetanephrine Antiserum**	1 x 5.25 ml	yellow	ready for use, from rabbit, yellow coloured
BA R-8301	STANDARD A	**Standard A**	1 x 12 ml	white	ready for use
BA R-8302	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA R-8303	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA R-8304	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA R-8305	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA R-8306	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
BA R-8312	ACYL-CONC	**Acylation Concentrate**	1 x 1.5 ml	dark grey	concentrated
BA R-8351	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA R-8352	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 - 500 µl; 3 ml; 10 ml
- 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
- Water (deionized, distilled, or ultra-pure)

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 500 x g to spin down adhering liquids.*

6.1 Preparation of reagents

Equalizing Reagent
The Equalizing Reagent has to be reconstituted with 10 ml water (deionized, distilled, or ultra-pure). 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 Concentrate to 3 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Use immediately!

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

6.2 Precipitation

1.	Pipette **100 µl** of **standards** and **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 min** at **3 000 x g**.
⚠	Take **100 µl** of the clear supernatant for the Metanephrine RIA and **25 µl** of the clear supernatant for the Normetanephrine RIA.

6.3 Metanephrine RIA

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

1.	Pipette **100 µl** of **water** (deionized, distilled, or ultra-pure) 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 min** 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 h** 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 h** (overnight) at **2 - 8 °C**. ***Alternatively** incubate for **2 h** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).*
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 and leave them upside for 2 minutes.
14.	**Count** all tubes for **1 min** in a gamma counter.

6.4 Normetanephrine RIA

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

1.	Pipette **25 µl** of **water** (deionized, distilled, or ultra-pure) into the **tubes** for the **NSB**.
2.	Pipette **25 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 maximum of only 3 minutes.***
5.	Mix thoroughly (vortex) and incubate for **15 min** at **RT** (20 - 25 °C).
6.	Pipette **50 µl** of **Normetanephrine Antiserum** into **all tubes *(except totals and NSB)***; mix thoroughly (vortex).
7.	Incubate for **1 h** at **RT** (20 - 25 °C).
8.	Pipette **50 µl** of the **^{125}I Normetanephrine** into **all tubes** and mix thoroughly (vortex).
9.	Cover tubes. Incubate for **15 - 20 h** (overnight) at **2 - 8 °C**. ***Alternatively** incubate for **2 h at RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).*
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 and leave them upside for 2 minutes.
14.	**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**
Normetanephrine (pg/ml)	0	48	160	480	1 600	4 800
Normetanephrine (pmol/l)	0	262	874	2 620	8 740	26 200
Metanephrine (pg/ml)	0	36	120	360	1 200	3 600
Metanephrine (pmol/l)	0	183	608	1 830	6 080	18 300
Conversion:	Normetanephrine (pg/ml) x 5.46 = Normetanephrine (pmol/l) 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.

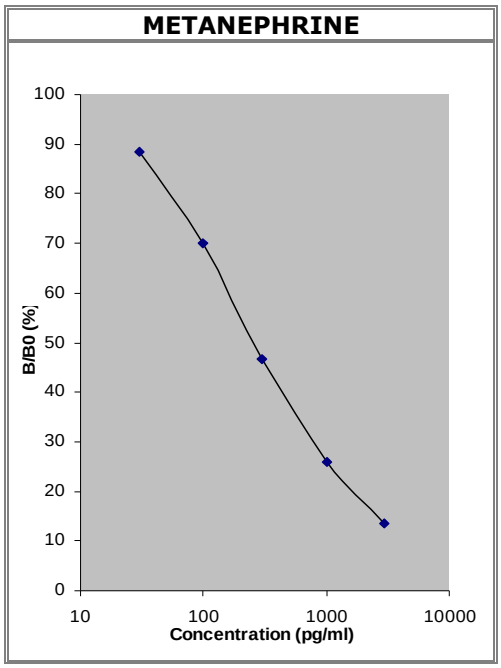
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Equalizing Reagent (BA R-0028) and have to be re-assayed.

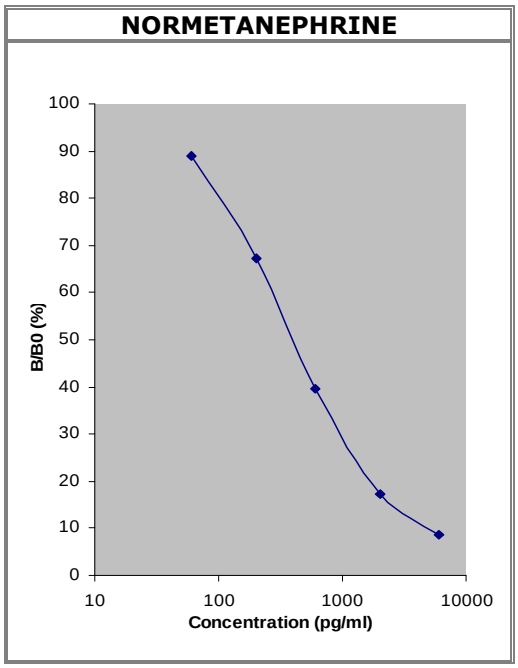
7.1 Quality control

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 calibration curves

⚠ Examples. Do not use for calculation!





8. Assay characteristics

		Metanephrine	Normetanephrine
Expected Reference Values	Plasma	< 90 pg/ml	< 180 pg/ml

		Metanephrine	Normetanephrine
Analytical Sensitivity (Limit of Detection)	Plasma	19 pg/ml	24 pg/ml

	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
Analytical Specificity (Cross Reactivity)	Derivatized Metanephrine	100	0.08
	Derivatized Normetanephrine	0.04	100
	3-Methoxytyramine.HCl	< 0.001	1.74
	Adrenaline	< 0.001	< 0.001
	Noradrenaline	< 0.001	< 0.001
	Dopamine.HCl	< 0.001	< 0.001
	Vanillic mandelic acid	< 0.001	< 0.001
	Homovanillic acid	< 0.001	< 0.001
	L-DOPA	< 0.001	< 0.001
	L-Tyrosin	< 0.001	< 0.001
	Tyramine.HCl	< 0.001	< 0.001
	Normetanephrine	< 0.001	< 0.001
	Acetaminophen	< 0.001	< 0.001

Precision							
Intra-Assay				**Inter-Assay**			
	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
Normetanephrine	1	234 ± 24	10	Normetanephrine	1	240 ± 20	8.3
	2	488 ± 43	8.7		2	518 ± 29	5.6
	3	1180 ± 93	7.9		3	1144 ± 71	6.2

Linearity			Range (pg/ml)	Serial dilution up to	Mean (%)
	Metanephrine	Plasma	25 - 2100	1: 65	91
	Normetanephrine	Plasma	40 - 6000	1: 129	100

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Metanephrine	Plasma	103	85 - 122	
	Normetanephrine	Plasma	107	95 - 119	

Method Comparison versus LC-MS/MS				
	Metanephrine	Plasma	LC-MS/MS = x – 13.2	r = 0.99; n = 50
	Normetanephrine	Plasma	LC-MS/MS = 1.2x – 29.6	r = 0.99; n = 50

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	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!