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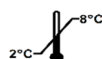


Instructions for use

Metanephrine Plasma ELISA **Fast Track**

REF

IB89181R



RUO

For research
use only –
Not for use
in diagnostic
procedures

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Related Products:

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

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of free metanephrine in plasma. The determination of metanephrine helps in the detection of paragangliomas and pheochromocytomas. Metanephrine (metadrenaline) is first extracted using an ion exchange matrix followed by an acylation process.

The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user.

This product is not intended to clinical diagnoses.

1.2 Background

Metanephrine and normetanephrine are the metabolites of the catecholamines epinephrine and norepinephrine, respectively [1]. Cells derived from neuroendocrine tumors (e.g. pheochromocytoma and paraganglioma) are known to produce catecholamines, which are secreted episodically via vesicles into the blood stream [2, 3]. But beside this, a small portion of the catecholamines is metabolized inside the tumor cells to the corresponding catecholamines metabolites – namely metanephrine, normetanephrine (and 3-methoxytyramine in the case of dopamine) – which are secreted at low levels continuously into the blood stream [4, 5].

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, 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 must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) The principles of Good Laboratory Practice (GLP) must be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
- (5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
- (6) Duplicate determination of sample is highly recommended.
- (7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
- (8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (10) A standard curve must be established for each run.
- (11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (12) 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.
- (13) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (14) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Rinse contaminated items before reuse.

- (15) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (17) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.
- (18) 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.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results. Commercially available synthetic metanephrine is always a mixture of the D- and L-form. This has important implications if synthetic metanephrine is used to enrich native samples. The antibody used in this kit has a specific D- and L-form recognition rate. Please contact the manufacturer for details in case synthetic metanephrine was used to enrich native samples.

2.2.1 Interfering substances and proper handling of specimens

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolytic samples (up to 0.5 mg/ml hemoglobin), icteric samples (up to 0.5 mg/ml bilirubin) and lipemic samples (up to 17 mg/ml triglycerides) have no influence on the assay results. If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

Medications like antihypertensive agents, antidepressants, antipsychotics, sympathomimetics and L-DOPA can influence plasma metanephrines levels. Caffeinated beverages, nicotine, and mood-enhancing drugs can also affect plasma metanephrines levels. In addition, stress and physical strain should be avoided shortly before sampling.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.




3. Storage and stability


Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0090	FOILS	Adhesive Foil – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	WASH-CONC 50x	Wash Buffer Concentrate – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	CONJUGATE	Enzyme Conjugate – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
BA E-0055	SUBSTRATE	Substrate – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	

BA E-0080	STOP-SOLN	Stop Solution – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0131	W ADR MN	Metanephrine Microtiter Strips – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant	
BA E-8110	MN-AS	Metanephrine Antiserum – ready to use
Content:	Rabbit anti-metanephrine antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-8327	ADJUST-BUFF	Adjustment Buffer – ready to use
Content:	TRIS buffer	
Volume:	1 x 10 ml/vial, yellow cap	
BA R-8312	ACYL-CONC	Acylation Concentrate – concentrated
Content:	Acylation reagent in DMSO	
Volume:	1 x 1.5 ml/vial, white cap	
Hazard pictograms:	 	
	GHS05 GHS08	
Signal word:	Danger	
Hazardous ingredients:	Succinic anhydride	
Hazard statements:	H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.	
Precautionary statements:	P260 Do not breathe mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310 Immediately call a POISON CENTER/doctor. P501 Dispose of contents/container to an authorised waste collection point.	
EUH-statements:	EUH071 Corrosive to the respiratory tract.	
BA R-8313	ASSAY-BUFF	Assay Buffer – ready to use
Content:	25% organic solvent	
Volume:	1 x 30 ml/vial, orange cap	
Hazard pictograms:		
	GHS02	
Signal word:	Warning	
BA R-8318	EXTRACT-PLATE 96	Extraction Plate – ready to use
Content:	1 x 96 well plate, precoated with ion-exchanger in a resealable pouch	
BA R-8325	CLEAN-CONC 25X	Cleaning Concentrate – concentrated 25x
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, green cap	
Hazard pictograms:		
	GHS05	
Signal word:	Danger	
Hazard statements:	H314 Causes severe skin burns and eye damage.	
Precautionary statements:	P280 Wear protective gloves, protective clothing, eye protection. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a doctor, a POISON CENTER. P501 Dispose of contents/container to an authorised waste collection point.	
BA R-8828	EQUA-REAG	Equalizing Reagent – ready to use
Content:	Human serum, negative for HIV I/II, HBsAg and HCV	
Description:	1 x 14 ml/vial, white cap	
Volume:	Species is human	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [pg/ml]	Concentration [pmol/l]	Volume/ Vial
			MN	MN	
BA E-8301	STANDARD A	white	0	0	4 ml
BA E-8302	STANDARD B	yellow	36	183	4 ml
BA E-8303	STANDARD C	orange	120	608	4 ml
BA E-8304	STANDARD D	blue	360	1,825	4 ml
BA E-8305	STANDARD E	grey	1,200	6,084	4 ml
BA E-8306	STANDARD F	black	3,600	18,252	4 ml
BA E-8351	CONTROL 1	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-8352	CONTROL 2	red			4 ml

Conversion: metanephrene [pg/ml] x 5.07 = metanephrene [pmol/l]

Content: Acidic buffer with non-mercury stabilizer, spiked with a defined quantity of metanephrene.

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 – 350 µl; 3 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer

5. Sample collection, handling and storage

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. When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay (see 2.2.1).

Storage: up to 3 days 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 and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the Extraction Plate and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

If the product is prepared in parts, unused wells in Extraction Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.

During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.

Storage: 2 months at 2 – 8 °C

Cleaning Buffer

Dilute the 20 ml Cleaning Concentrate **CLEAN-CONC** **25X** with water to a final volume of 500 ml.

Storage: 2 months at 2 – 8 °C

Acylation Solution

⚠ As the Acylation Solution is only **stable for a maximum of 3 minutes**, it should not be prepared before starting the assay. Therefore, its preparation is described in the protocol in chapter 6.3, step 3. Discard after use!

Metanephrine Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Extraction Plate

In rare cases residues of the cation exchanger can be seen in the wells as small, black dots or lines. These residues do not influence the quality of the product.

6.2 Preparation of samples – Extraction

The following extraction procedure can be run with 200 µl or 250 µl of plasma sample.

The procedure for 250 µl plasma is highlighted in grey and italicised and may be used in case higher supernatant volumes for pipetting to the subsequent ELISA are preferred.

The ELISA procedure itself is not affected by this alternative protocol.

1.	Pipette 20 µl of standards and controls into the respective wells of the EXTRACT-PLATE 96 . <i>Alternatively pipette 25 µl of standards and controls.</i>
2.	Add 20 µl STANDARD A to all wells intended for the plasma samples . <i>Alternatively add 25 µl STANDARD A.</i>
3.	Add 200 µl of EQUA-REAG to the wells with standards and controls . <i>Alternatively add 250 µl of EQUA-REAG.</i>
4.	Pipette 200 µl of plasma samples to the respective wells. <i>Alternatively pipette 250 µl of plasma samples.</i>
5.	Incubate plate for 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.	Pipette 250 µl of ASSAY-BUFF into all wells. Incubate the plate for 5 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8.	Wash the plate 3 times by adding 350 µl of Cleaning Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
9.	Pipette 100 µl of ELUTION-BUFF into all wells. <i>Alternatively pipette 125 µl of ELUTION-BUFF.</i> <i>Please note: The colour changes caused by the elution buffer can vary between standards and samples.</i>
10.	Cover plate with FOILS . Incubate 15 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). ⚠ Remove the FOILS . Do not decant the supernatant thereafter! The following volume of the supernatant is needed for the subsequent ELISA: Metanephrine 50 µl

6.3 Metanephrine ELISA

1.	Pipette 25 µl of ADJUST-BUFF into all wells of the Metanephrine Microtiter Strips W ADR MN .
2.	Pipette 50 µl of the extracted standards, controls and samples into the respective wells. <i>Please hold the Extraction Plate at a slight angle in order to facilitate this pipetting step.</i>
3.	Preparation of Acylation Solution : Pipette 80 µl ACYL-CONC to 3 ml water and mix thoroughly.
4.	Pipette 25 µl of the freshly prepared Acylation Solution into all wells.
5.	Incubate for 15 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
6.	Pipette 50 µl of the Metanephrine Antiserum MN-AS into all wells.
7.	Cover the plate with FOILS , shake for 1 min at RT (20 – 25 °C) on a shaker and incubate for 15 – 20 h (overnight) at 2 – 8 °C without shaking.
8.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 4 times by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
9.	Pipette 100 µl of the CONJUGATE into all wells.
10.	Incubate for 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
11.	Discard or aspirate the contents of the wells. Wash the plate 4 times by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
12.	Pipette 100 µl of the SUBSTRATE into all wells and incubate for 20 – 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
13.	Add 100 µl of the STOP-SOLN to all wells and shake the microtiter plate shortly.
14.	Read the absorbance of the solution in the wells within 10 min, using a microplate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Metanephrine
	15.1 – 3,600 pg/ml

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 pg/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

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

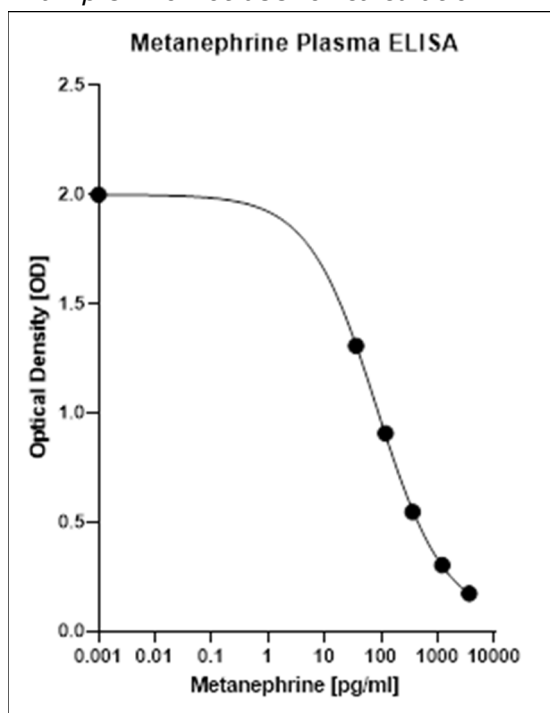
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with the included Equalizing Reagent **EQUA-REAG** and must be re-assayed.

Conversion:

metanephrine [pg/ml] x 5.07 = metanephrine [pmol/l]

7.1 Typical standard curve

⚠ Example: Do not use for calculation!



8. Control samples

The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	
	Metanephrine
Limit of Blank (LOB)	9.9 pg/ml
Limit of Detection (LOD)	14.9 pg/ml
Limit of Quantification (LOQ)	15.1 pg/ml

Analytical Specificity (Cross Reactivity)	
Substance	Cross Reactivity [%]
	Metanephrine
Metanephrine	100
Normetanephrine	0.05
3-Methoxytyramin	< 0.01
Adrenaline	< 0.01
Noradrenaline	< 0.01
Dopamin	< 0.01
Vanillic mandelic acid	< 0.01
Homovanillic acid	< 0.01
L-DOPA	< 0.01
L-Tyrosin	< 0.01
Tyramine	< 0.01
Acetaminophen	< 0.01

Precision							
Intra-Assay				Inter-Assay			
	Sample	Mean [pg/ml]	CV [%]		Sample	Mean [pg/ml]	CV [%]
Metanephrine	1	66.3	11.4	Metanephrine	1	67.8	17.6
	2	122	13.5		2	134	12.7
	3	308	10.6		3	319	11.0
	4	783	9.2		4	847	7.5

Lot-to-Lot			
	Sample	Mean ± SD [pg/ml]	CV [%]
Metanephrine (n = 6)	1	97.7 ± 16.5	16.9
	2	870 ± 117	13.5

Recovery was determined according to the CLSI standard EP 34 1st ed.

Recovery			
	Range [pg/ml]	Mean [%]	Range [%]
Metanephrine	20.9 – 1,291	102	90 – 109

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Metanephrine	1:64	107	101 – 124

Method Comparison: ELISA vs. LC-MS/MS [6]	
Metanephrine	$y = 0.91x + 1.8$; $r^2 = 0.96$; $n = 46$

9.2 Metrological Traceability

The values assigned to the standards and controls of the Metanephrine Plasma ELISA ^{Fast Track} are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	
	Uncertainty [%]
Metanephrine	2.0

Metanephrine Plasma ELISA ^{Fast Track}	
	Expanded Uncertainty [%] $k = 2^*$
Metanephrine	25.7

*This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature













1. Lee, S.M., et al., *Development and validation of liquid chromatography-tandem mass spectrometry method for quantification of plasma metanephrines for differential diagnosis of adrenal incidentaloma*. Ann Lab Med, 2015. **35**(5): p. 519-22.
2. Peaston, R.T., et al., *Performance of plasma free metanephrines measured by liquid chromatography-tandem mass spectrometry in the diagnosis of pheochromocytoma*. Clin Chim Acta, 2010. **411**(7-8): p. 546-52.
3. de Jong, W.H., et al., *Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors*. J Clin Endocrinol Metab, 2009. **94**(8): p. 2841-9.
4. Boot, C., et al., *Single-centre study of the diagnostic performance of plasma metanephrines with seated sampling for the diagnosis of phaeochromocytoma/paraganglioma*. Ann Clin Biochem, 2017. **54**(1): p. 143-148.
5. Shen, Y., et al., *A simple and robust liquid chromatography tandem mass spectrometry assay for determination of plasma free metanephrines and its application to routine clinical testing for diagnosis of pheochromocytoma*. Biomed Chromatogr, 2019. **33**(10): p. e4622.
6. de Jong, W.H., et al., *Plasma free metanephrine measurement using automated online solid-phase extraction HPLC tandem mass spectrometry*. Clin Chem, 2007. **53**(9): p. 1684-93.

For updated literature or any other information please contact your local supplier.

11. Changes

Version	Release Date	Chapter	Change
20.0-r	2022-03-25	All	<ul style="list-style-type: none"> - The alternative version, 2 h at RT incubation with antiserum, was removed - Sample stability (chapter 5) changed - LOB and Lot to Lot were added to the assay characteristics (chapter 9.1) - Metrological traceability was added (chapter 9.2) - References/Literature was updated (chapter 10)
21.0-r	2023-09-18	4.1 9.1 9.2 10	<ul style="list-style-type: none"> - Hazard labelling updated according to SDS - Recovery updated - Editorial changes - References updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!