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Instructions for use

2-MET Urine ELISA Fast Track

REF **IB89186R**

2°C – 8°C

Σ 2 x 96

RUO

For research use only – Not for use in diagnostic procedures

Table of contents

Related Products:

- Metanephrine Urine ELISA Fast Track
- Normetanephrine Urine ELISA Fast Track

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Metanephrine and Normetanephrine in urine. During the sample preparation Metanephrine (Metadrenaline) and Normetanephrine (Normetadrenaline) are quantitatively acylated.
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. They are metabolized to Vanillylmandelic acid or excreted with the urine.
As catecholamine secretion from neuroendocrine cells might show high variations, urine samples collected over a period of 24 hours are used to average these fluctuations.

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 H_2SO_4. 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.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

⚠*The anti-Metanephrine antibodies used in this test kit only recognise the biologically relevant L-forms of Metanephrine. Commercially available synthetic 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 Metanephrine is used to enrich native samples. As only about 50% of the synthetic Metanephrine – the L-portion – will be detected by use of this kit, spiked samples will be underestimated. Therefore, native samples containing solely the L-form should be used.*

2.2.1 Interfering substances and proper handling of specimens

24-hour urine

Please note the sample collection! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug and food interferences

There are no known substances (drugs) which ingestion interferes with the measurement of (Nor-)metanephrine level in the sample.

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 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-0045	**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	**Ш** **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-0231	**Ш** **NAD** **NMN**	**Normetanephrine Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable yellow pouch with desiccant	

BA E-8410	**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 12 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-8510	**NMN-AS**	**Normetanephrine Antiserum** – ready to use
Content:	Rabbit anti-normetanephrine antibody in buffer with proteins and non-mercury preservative, yellow coloured	
Volume:	1 x 12 ml/vial, yellow cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA R-0012	**ACYL-CONC**	**Acylation Concentrate** – concentrated
Content:	Concentrated acylation reagent	
Volume:	1 x 0.5 ml/vial, white cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P261 Avoid breathing mist/vapours/spray. P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P362+P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/container to an authorised waste collection point.	
BA R-0075	**ACYL-DILUENT**	**Acylation Diluent** – ready to use
Content:	Dimethylsulfoxide	
Volume:	1 x 3.5 ml/vial, white cap	
BA R-8611	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	TRIS buffer	
Volume:	1 x 30 ml/vial, white cap	
BA R-8619	**HCL**	**Hydrochloric Acid** – ready to use
Content:	0.25 M hydrochloric acid, yellow coloured	
Volume:	1 x 30 ml/vial, green cap	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [ng/ml] (= µg/l)		Concentration [nmol/l]		Volume/ Vial
			MN	NMN	MN	NMN	
BA R-8601	STANDARD A	white	0	0	0	0	4 ml
BA R-8602	STANDARD B	yellow	20	30	101	164	4 ml
BA R-8603	STANDARD C	orange	60	90	304	491	4 ml
BA R-8604	STANDARD D	blue	200	300	1,014	1,638	4 ml
BA R-8605	STANDARD E	grey	600	900	3,042	4,914	4 ml
BA R-8606	STANDARD F	black	2,000	3,000	10,140	16,380	4 ml
BA R-8651	CONTROL 1	green	Refer to QC-Report for expected value and acceptable range.				4 ml
BA R-8652	CONTROL 2	red					4 ml

Conversion: metanephrine [ng/ml] x 5.07 = metanephrine [nmol/l]
normetanephrine [ng/ml] x 5.46 = normetanephrine [nmol/l]

Content: Acidic buffer with non-mercury preservatives, spiked with defined quantity of metanephrine and normetanephrine.

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)
- Reaction tubes, at least 3 ml, Polypropylene/Polystyrol

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 600 µl; 1.2 – 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
- Vortex mixer
- Temperature controlled water bath (90 °C) or similar heating device

⚠ *The assay can be performed with or without shaking. If a microtiter plate shaker is used, it should have the following characteristics: shaking amplitude 3 mm; approx. 600 rpm.*

5. Sample collection, handling and storage

24-hour urine, collected in a bottle containing 10 – 15 ml of 6 M HCl, should be used.
Determine the total volume of urine excreted during a period of 24 h for calculation of the results.
Storage: up to 5 days at 2 – 8 °C, for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the reaction tubes 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 sample preparation (hydrolysis and acylation) is identical for both the Metanephrine and Normetanephrine assay and has to be done only once.*

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.

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

Acylation Solution

⚠ *Before preparing the Acylation Solution make sure that the* ***ACYL-DILUENT*** *(BA R-0075) has reached room temperature (≥ 20 °C) and forms a homogenous, crystal-free solution.*

Dilute the **ACYL-CONC** (BA R-0012) 1 + 60 with **ACYL-DILUENT** (BA R-0075) in a glass or polypropylene-vial.

ACYL-CONC (BA R-0012)	10 µl	15 µl	25 µl	50 µl
ACYL-DILUENT (BA R-0075)	600 µl	900 µl	1.5 ml	3 ml

⚠ *The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!*

Metanephrine Microtiter Strips and Normetanephrine 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.

6.2 Preparation of samples

Hydrolysis

1.	Pipette **25 µl** of **standards**, **controls,** and **urine samples** into the respective reaction tubes.
2.	Add **250 µl** **HCL** to all tubes.
3.	Mix thoroughly (vortex) and hydrolyze for **30 min** at **90 °C**.
4.	Cool down the tubes to room temperature.

Acylation

1.	Pipette **250 µl** of **ACYL-BUFF** into all tubes.
2.	Add **25 µl** of **Acylation Solution** *(refer to 6.1)* to all tubes.
3.	Mix thoroughly (vortex) and acylate for **15 min** at **RT** (20 – 25 °C).
4.	Add **2.5 ml water** (deionized, distilled, or ultra-pure) to all tubes.
⚠	Take **25 µl** of the acylated **standards**, **controls and urine samples** for the **Metanephrine ELISA and Normetanephrine ELISA**.

6.3 Metanephrine ELISA

The usage of a shaker is not mandatory. The alternative protocol without shaker is highlighted in italic and shaded in grey.

1.	Pipette **25 µl** of the **acylated standards, controls and samples** into the appropriate wells of the **Metanephrine Microtiter Strips** **Ш** **ADR** **MN**.
2.	Pipette **100 µl** of the **MN-AS** into all wells.
3.	Incubate **30 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *shake the* ***Metanephrine Microtiter Strips*** **Ш** **ADR** **MN** *shortly by hand and incubate for* ***1 h at RT*** *(20 – 25 °C).*
4.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **CONJUGATE** into all wells.
6.	Incubate for **15 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *incubate for* ***15 min at RT*** *(20 – 25 °C).*
7.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **SUBSTRATE** into all wells.
9. ⚠	Incubate for **15 ± 2 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *incubate for* ***15 min ± 2 at RT*** *(20 – 25 °C).* ***Avoid exposure to direct sunlight!***
10.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
11.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

6.5 Normetanephrine ELISA

The usage of a shaker is not mandatory. The alternative protocol without shaker is highlighted in italic and shaded in grey.

1.	Pipette **25 µl** of the **acylated standards, controls and samples** into the appropriate wells of the **Normetanephrine Microtiter Strips** **Ш** **NAD** **NMN**.
2.	Pipette **100 µl** of the **NMN-AS** into all wells.
3.	Incubate **30 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *shake the* ***Normetanephrine Microtiter Strips*** **Ш** **NAD** **NMN** *shortly by hand and incubate for* ***1 h at RT*** *(20 – 25 °C).*
4.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **CONJUGATE** into all wells.
6.	Incubate for **15 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *incubate for* ***15 min at RT*** *(20 – 25 °C).*
7.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **SUBSTRATE** into all wells.
9.	Incubate for **15 ± 2 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *incubate for* ***15 min ± 2 at RT*** *(20 – 25 °C).* ⚠ ***Avoid exposure to direct sunlight!***
10.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
11.	**Read** the absorbance of the solution in the wells within 10 minutes, 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	Normetanephrine
	10.5 – 2,000 ng/ml	16.2 – 3,000 ng/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 ng/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.
The amount of analyte excreted per day [µg/day] is calculated according to:

concentration of the sample [in µg/l] x volume of urine excreted per day [in l/day]

Example

The concentration of the sample read from the curve is 125 µg/l. The amount of urine collected during 24 hours is 1.3 l. Then the amount of analyte excreted during one day would be:

125 µg/l x 1.3 l/day = 162.5 µg/day

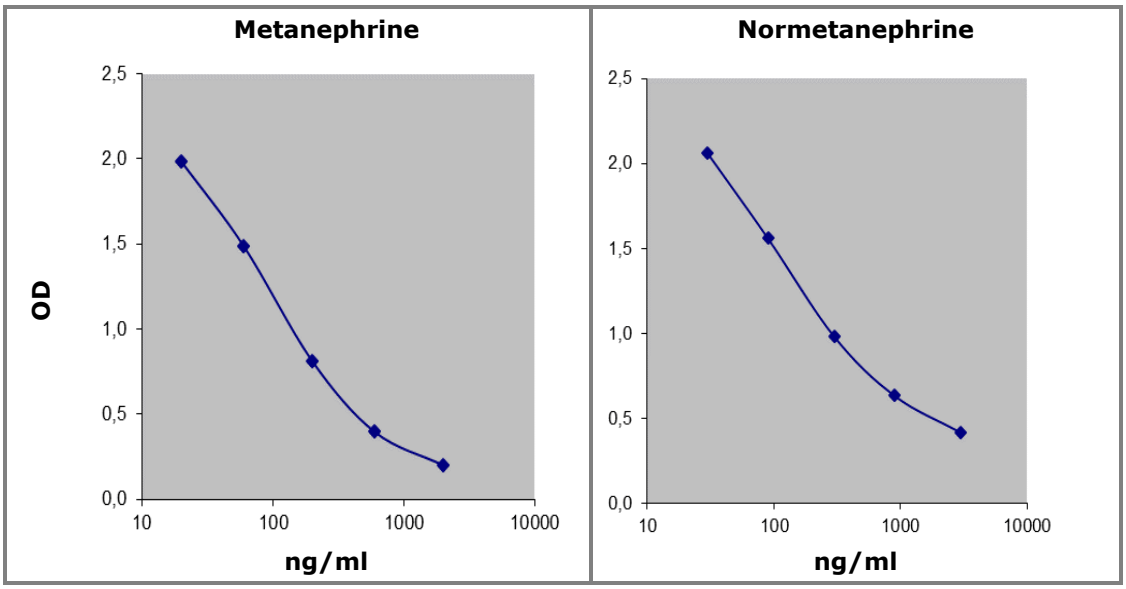
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and must be re-assayed.

Conversion:

Metanephrine [ng/ml] x 5.07 = Metanephrine [nmol/l]
Normetanephrine [ng/ml] x 5.46 = Normetanephrine [nmol/l]

7.1 Typical standard curve

⚠*Examples: 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	Normetanephrine
Limit of Blank (LOB)	6.0 ng/ml	10.4 ng/ml
Limit of Detection (LOD)	8.6 ng/ml	14.7 ng/ml
Limit of Quantification (LOQ)	10.5 ng/ml	16.2 ng/ml

Analytical Specificity (Cross Reactivity)		
Substance	**Cross Reactivity [%]**	
	Metanephrine	Normetanephrine
Derivatized Metanephrine	100	0.11
Derivatized Normetanephrine	0.15	100
Derivatized 3-methoxytyramine	< 0.01	0.19
Adrenaline	3.3	< 0.01
Noradrenaline	< 0.01	0.64
Dopamine	< 0.01	< 0.01
Vanillic mandelic acid, L-Dopa, Homovanillic acid, L-Tyrosin, Tyramin	< 0.01	< 0.01

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range [ng/ml]	CV [%]		Sample	Range [ng/ml]	CV [%]
Metanephrine	1	34.4 ± 3.1	9	Metanephrine	1	32.8 ± 5.4	16
	2	59.8 ± 5.1	9		2	57.2 ± 9.0	16
	3	141 ± 13.8	10		3	144 ± 25.0	17
	4	575 ± 71.4	12		4	394 ± 64.1	16
Normetanephrine	1	53.9 ± 7.1	13	Normetanephrine	1	52.5 ± 10.7	20
	2	116 ± 12.3	11		2	103 ± 15.2	15
	3	322 ± 28.1	9		3	276 ± 38.7	14
	4	1,121 ± 128	11		4	738 ± 83.0	11

Recovery			
	Range [ng/ml]	Mean [%]	Range [%]
Metanephrine	20.2 – 1,484	97	85 – 113
Normetanephrine	26.5 – 3,124	100	93 – 111

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Metanephrine	1:64	102	94 – 115
Normetanephrine	1:64	101	90 – 113

Method Comparison: ELISA vs. HPLC*		
Metanephrine	HPLC = 0.9 ELISA – 0.8	r = 0.99; n = 40
Normetanephrine	HPLC = 0.9 ELISA + 0.6	r = 0.99; n = 40

* The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. Please take in mind, that the UK control values are the mean of about 40 different HPLC users and contain always one pathological sample per sending.

10. References/Literature

(1) Parrott et al. Urinary corticosterone and normetanephrine levels after voluntary wheel and forced treadmill running in the db/db mouse. Journal of Diabetes Mellitus, 1(4):71-78 (2011)
(2) Petramala et al. Multiple Catecholamine-Secreting Paragangliomas: Diagnosis after Hemorrhagic Stroke in a Young Woman. Endocrine Practice, 14(3):340-346 (2008)
(3) Sato et al. Central control of bone remodeling by neuromedin U. Nature Medicine, 13:1234-1240 (2007)

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

11. Changes

Version	Release Date	Chapter	Change
17.0-r	2023-10-24	4.1 4.1 4.3	- BA D-0023 Reaction Tubes removed - Hazard labelling updated according to SDS - Reaction Tubes listed as additional material

Symbols:

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