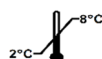


Instructions for use

Glutamate ELISA

Please use only the valid version of the Instructions for Use provided with the kit

REF**BA E-2400R****RUO**

For research
use only –
Not for use
in diagnostic
procedures

Table of contents

1.	Introduction	3
1.1	Intended use and principle of the test	3
1.2	Background	3
2.	Procedural cautions, guidelines, warnings and limitations	3
2.1	Procedural cautions, guidelines and warnings	3
2.2	Limitations	4
2.2.1	Interfering substances and proper handling of specimens	4
2.2.2	Drug and food interferences	4
2.2.3	High-Dose-Hook effect	4
3.	Storage and stability	4
4.	Materials	4
4.1	Contents of the kit	4
4.2	Calibration and Controls	6
4.3	Additional materials required but not provided in the kit	6
4.4	Additional equipment required but not provided in the kit	6
5.	Sample collection, handling and storage	6
6.	Test procedure	6
6.1	Preparation of reagents and further notes	7
6.2	Preparation of samples	7
6.3	Preparation of samples – Extraction	7
6.4	Derivatization	7
6.5	Glutamate ELISA	8
7.	Calculation of results	8
7.1	Typical standard curve	9
8.	Control samples	9
9.	Assay characteristics	9
9.1	Performance data	9
9.2	Metrological Traceability	10
10.	References/Literature	10
11.	Changes	11

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-glutamate in urine and various biological samples. After extraction and derivatisation Glutamate is quantitatively determined by ELISA. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized analyte concentrations in the 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

Glutamate, also known as glutamic acid, is one of the most important excitatory neurotransmitter in the central nervous system (CNS). It is released presynaptically and it binds postsynaptically to specific receptors for glutamate. The enzyme glutamic acid decarboxylase is able to convert L-glutamate in the CNS by decarboxylation to γ -aminobutyric acid (GABA), which acts as an inhibitory neurotransmitter.

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.

2.2 Limitations

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

2.2.1 Interfering substances and proper handling of specimens

Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer.

A pH of 5.0 during the extraction is mandatory.

Urine

Please note the sample preparation stabilization of the urine sample! It cannot be excluded that high acid concentrations lead to incorrect results. Up to 20 µl 6 M HCl per 1 ml urine no influence on the results was observed.

2.2.2 Drug and food interferences

There are no known substances (drugs, food) which ingestion interferes with the measurement of glutamate 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 D-0024	REAC-PLATE	Reaction Plate – ready to use
Content:	1 x 96 well plate, empty, in a resealable pouch	
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-2410	AS GLUT	Glutamate Antiserum – ready to use
Content:	Rabbit anti-glutamate antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	

BA E-2413	ASSAY-BUFF	Assay Buffer – ready to use
Content:	Buffer with alkaline pH	
Volume:	1 x 20 ml/vial, yellow cap	
Hazard pictograms:	 	
	GHS08 GHS07	
Signal word:	Danger	
Hazardous ingredients:	Boric acid	
Hazard statements:	H360FD May damage fertility. Suspected of damaging the unborn child.	
Precautionary statements:	P201 Obtain special instructions before use. P280 Wear protective gloves, protective clothing, eye protection, face protection. P308+P313 IF exposed or concerned: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
Additional statements:	Restricted to professional users.	
BA E-2428	EQUA-REAG	Equalizing Reagent – lyophilized
Content:	Lyophilized protein	
Volume:	1 vial, brown cap	
Description:	Species is bovine	
BA E-2431	 GLUT	Glutamate Microtiter Strips – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	
BA E-2442	EXTRACT-PLATE 	Extraction Plate – ready to use
Content:	2 x 48 well plate, precoated with cation exchanger in a resealable pouch	
BA E-2446	D-REAGENT	D-Reagent – ready to use
Content:	Crosslinking agent in dimethylsulfoxide	
Volume:	1 x 3 ml/vial, white cap	
Hazard pictograms:		
	GHS07	
Signal word:	Warning	
Hazardous ingredients:	Glutaraldehyde	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P261 Avoid breathing mist/vapours/spray. P280 Wear protective gloves. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-2458	Q-BUFFER	Q-Buffer – ready to use
Content:	TRIS buffer	
Volume:	1 x 20 ml/vial, white cap	
BA E-2460	DILUENT	Diluent – ready to use
Content:	Buffer with sodium acetate	
Volume:	1 x 20 ml/vial, green cap	

BA E-2787	NAOH	NaOH – ready to use
Content:	Sodium hydroxide solution	
Volume:	1 x 2 ml/vial, purple cap	
Hazard pictograms:		
	GHS07	
Signal word:	Warning	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/Cap	Concentration [µg/ml]	Concentration [µmol/l]	Volume/Vial
BA E-2401	STANDARD A	white	0	0	4 ml
BA E-2402	STANDARD B	yellow	0.6	4.08	4 ml
BA E-2403	STANDARD C	orange	2	13.6	4 ml
BA E-2404	STANDARD D	blue	6	40.8	4 ml
BA E-2405	STANDARD E	grey	20	136	4 ml
BA E-2406	STANDARD F	black	60	408	4 ml
BA E-2451	CONTROL 1	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-2452	CONTROL 2	red			4 ml

Conversion: glutamate [µg/ml] x 6.8 = glutamate [µmol/l]

Content: Acidic buffer with non-mercury preservatives, spiked with a defined quantity of glutamate.

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 10 – 100 µl; 12.5 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

Various biological samples can be used for L-Glutamate determination. The assay was validated for human urine samples.

Urine

Spontaneous urine (second morning urine) stabilized with 10 µl 6 M HCl per 1 ml of urine sample should be used. The measurement results are related to the creatinine content of the sample.

Storage: up to 6 hours at 18 – 25 °C; up to 14 days at 2 – 8 °C; up to 6 months at < -15 °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 Extraction Plate, Reaction 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 Reaction and 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

Equalizing Reagent

Reconstitute the **EQUA-REAG** with **12.5 ml** of **ASSAY-BUFF**.

Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max. 2 months at < -15 °C and may be thawed only once.

D-Reagent

The **D-REAGENT** has a freezing point of 18.5 °C. Make sure that the **D-REAGENT** has reached room temperature and forms a homogeneous, crystal-free solution.

Glutamate 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

The Glutamate ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

- Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A **pH of 5.0** during the extraction is mandatory.
- It is advisable to perform a **Proof of Principle** to determine the recovery of glutamate from the samples. Prepare a stock solution of glutamate. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The sample volume determines the sensitivity of this test. Determine the sample volume needed to determine glutamate in your sample by testing different amounts of sample volumes.
- If a sample volume **< 100 µl** is used, water (deionized, distilled, or ultra-pure) has to be added to a final volume of **100 µl**.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Preparation of samples – Extraction

1.	Pipette 100 µl of the standards, controls and urine samples into the appropriate wells of the EXTRACT-PLATE 48 .
2.	Add 100 µl of the DILUENT to all wells. Cover plate with FOILS and shake for 10 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
3.	Use 25 µl for the subsequent derivatization!

6.4 Derivatization

1.	Pipette 25 µl of the extracted standards, controls and urine samples into the appropriate wells of the REAC-PLATE .
2.	Pipette 10 µl of NAOH into all wells.
3.	Pipette 50 µl of the Equalizing Reagent into all wells.
4.	Pipette 10 µl of the D-REAGENT into all wells.
5.	Cover plate with FOILS and shake for 2 h at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
6.	Pipette 75 µl of the Q-BUFFER into all wells.
7.	Shake for 10 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
8.	Use 25 µl for the ELISA!

6.5 Glutamate ELISA

1.	Pipette 25 µl of the prepared standards, controls and urine samples into the appropriate wells of the Glutamate Microtiter Strips W GLUT .
2.	Pipette 50 µl of the AS GLUT into all wells and mix shortly.
3.	Cover plate with FOILS and incubate for 15 – 20 h (overnight) at 2 – 8 °C .
4.	Remove the foil. 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 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells and 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 and incubate for 20 – 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
9.	Add 100 µl of the STOP-SOLN to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	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	Glutamate	
	Urine	0.26 – 60 µg/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 µg/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 (100 µl undiluted sample used) and controls can be read directly from the standard curve.

⚠ In case < 100 µl sample volume was used, concentrations of the samples taken from the standard curve have to be multiplied by a correction factor:

$$\text{Correction factor} = \frac{100 \text{ µl (volume of standards)}}{\text{sample volume (µl)}}$$

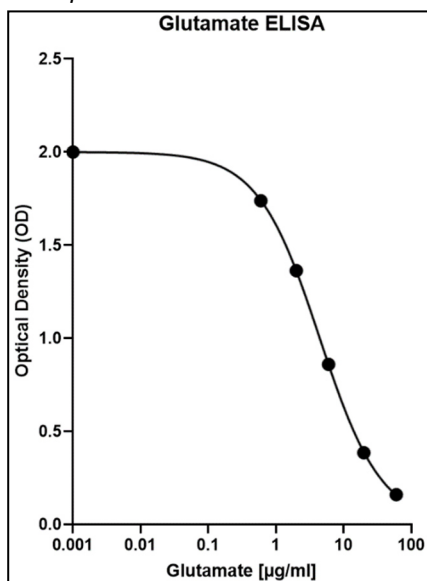
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and must be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.

Conversion:

$$\text{Glutamate [µg/ml]} \times 6.8 = \text{Glutamate [µmol/l]}$$

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

Various biological samples can be used for L-Glutamate determination. The assay was validated for human urine samples.

9.1 Performance data

Analytical Sensitivity	
	Glutamate
Limit of Blank (LOB)	0.11 µg/ml
Limit of Detection (LOD)	0.17 µg/ml
Limit of Quantification (LOQ)	0.26 µg/ml

Analytical Specificity (Cross Reactivity)	
Substance	Cross Reactivity [%]
	Glutamate
L-Glutamine	< 0.4
Glycine	< 0.4
β-Alanine	< 0.4
L-Alanine	< 0.4
L-Aspartic Acid	< 0.4
GABA	< 0.4
5-Amino-n-valeric Acid	< 0.4

Precision							
Intra-Assay				Inter-Assay			
Sample	n	Mean ± SD [µg/ml]	CV [%]	Sample	n	Mean ± SD [µg/ml]	CV [%]
1	10	0.8 ± 0.1	10.8	1	13	1.7 ± 0.24	14.3
2	10	1.3 ± 0.1	8.7	2	14	5.0 ± 0.57	11.4
3	10	2.2 ± 0.1	6.3	3	14	10.6 ± 0.73	6.9
4	10	4.8 ± 0.2	4.0	4	13	3.0 ± 0.43	14.2
5	10	12.5 ± 0.6	4.6	5	14	5.6 ± 0.71	12.5
6	10	39.7 ± 2.2	5.6	6	14	10.0 ± 0.87	8.7

Lot-to-Lot			
	Sample	Mean $\pm$ SD [$\mu\text{g/ml}$]	CV [%]
Glutamate in urine (n=3)	1	13.3 $\pm$ 1.2	9.4
Glutamate in artificial matrix (n = 3)	2	5.0 $\pm$ 0.5	10.1

Recovery			
	Range [$\mu\text{g/ml}$]	Mean [%]	Range [%]
Urine	1.25 – 41.0	102	97 – 108

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Urine	1:64	105	94 – 113

9.2 Metrological Traceability

The values assigned to the standards and controls of the Glutamate ELISA are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	
	Uncertainty [%]
Glutamate	1.4

Glutamate ELISA	
Concentration [$\mu\text{g/ml}$]	Expanded Uncertainty [%] $k = 2^*$
1.7	28.7
5	23.0
10.6	14.1

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

10. References/Literature

1. Bieger, W.P., *NeuroStress Guide*. 2011.
2. Bustillo, J.R., *Use of proton magnetic resonance spectroscopy in the treatment of psychiatric disorders: a critical update*. Dialogues Clin Neurosci. 2013. **15**(3): p. 329-37.
3. Duman, R.S., G. Sanacora, and J.H. Krystal, *Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments*. Neuron, 2019. **102**(1): p. 75-90.
4. Femenia, T., et al., *Dysfunctional hippocampal activity affects emotion and cognition in mood disorders*. Brain Res, 2012. **1476**: p. 58-70.
5. Flasnoecker, M., *Reise aus dem Stress - Körper, Geist und Psyche stärken*. 2015: W. Zuckschwerdt Verlag GmbH.
6. Frisardi, V., F. Panza, and A.A. Farooqui, *Late-life depression and Alzheimer's disease: the glutamatergic system inside of this mirror relationship*. Brain Res Rev, 2011. **67**(1-2): p. 344-55.
7. Gao, S.F. and A.M. Bao, *Corticotropin-releasing hormone, glutamate, and gamma-aminobutyric acid in depression*. Neuroscientist, 2011. **17**(1): p. 124-44.
8. Harris, R.E. and D.J. Clauw, *Imaging central neurochemical alterations in chronic pain with proton magnetic resonance spectroscopy*. Neurosci Lett, 2012. **520**(2): p. 192-6.
9. Kendell, S.F., J.H. Krystal, and G. Sanacora, *GABA and glutamate systems as therapeutic targets in depression and mood disorders*. Expert Opin Ther Targets, 2005. **9**(1): p. 153-68.
10. Krystal, J.H., et al., *Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments*. Mol Psychiatry, 2002. **7 Suppl 1**: p. S71-80.
11. Lener, M.S., et al., *Glutamate and Gamma-Aminobutyric Acid Systems in the Pathophysiology of Major Depression and Antidepressant Response to Ketamine*. Biol Psychiatry, 2017. **81**(10): p. 886-897.
12. Sanacora, G., G. Treccani, and M. Popoli, *Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders*. Neuropharmacology, 2012. **62**(1): p. 63-77.
13. Strienz, J., *Nebennierenunterfunktion - Stress stört die Hormonbalance*. 3 ed. 2019: W. Zuckschwerdtverlag München.

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

11. Changes

Version	Release Date	Chapter	Change
17.0-r	2024-05-28	4.1	- Hazard labelling updated according to SDS
		9.1	- Lot-to-Lot added
		9.2	- Chapter Metrological Traceability added
18.0-r	2024-09-30	9.1	- Lot-to-lot 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!