Instructions for use

Glutamate ELISA

REF **BA E-2400**

Σ 96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

Glutamate ELISA

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Glutamate in urine, plasma and serum samples.

After extraction and derivatisation Glutamate is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated analyte concentrations in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. When 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. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standards.

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 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 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 microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(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 wells 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 provided with the kit.
(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) 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.
(17) 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.
(18) 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. Wash contaminated objects before reusing them.
(19) For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.

(20) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(21) Kit reagents must be regarded as hazardous waste and disposed of 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

Serum/Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence interfering factors are not extracted quantitatively.

2.2.2 Drug interferences

There are no known substances (drugs) 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 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. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA D-0024 REAC-PLATE **Reaction Plate** - Ready to use
Contents: 1 x 96 well plate, empty in a resealable pouch

BA E-2442 EXTRACT-PLATE 48 **Extraction Plate** - Ready to use
Contents: 2 x 48 well plate, precoated with cation exchanger in a resealable pouch

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

BA E-0040 CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use

Contents: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

BA E-2431 MTP GLUT **Glutamate Microtiter Strips** - Ready to use

Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable foil pouch with desiccant

BA E-2410 AS GLUT **Glutamate Antiserum** - Ready to use

Contents: Rabbit anti- glutamate antibody, blue coloured

Volume: 1 x 6 ml/vial, blue cap

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	light yellow	0.6	4.08	4 ml
BA E-2403	STANDARD C	orange	2	13.6	4 ml
BA E-2404	STANDARD D	dark blue	6	40.8	4 ml
BA E-2405	STANDARD E	light grey	20	136	4 ml
BA E-2406	STANDARD F	black	60	408	4 ml
BA E-2451	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-2452	CONTROL 2	dark red			4 ml

Conversion: Glutamate (µg/ml) x 6.8 = Glutamate (µmol/l)

Contents: Acidic buffer with non-mercury preservative, spiked with defined quantity of Glutamate

BA E-2413 ASSAY-BUFF **Assay Buffer** - Ready to use

Contents: Buffer with alkaline pH

Volume: 1 x 20 ml/vial, yellow cap

BA E-2428 EQUA-REAG **Equalizing Reagent** - Lyophilized

Contents: Lyophilized protein

Volume: 1 vial, brown cap

BA E-2446 D-REAGENT **D-Reagent** - Ready to use

Contents: Crosslinking agent in dimethylsulfoxide

Volume: 1 x 4 ml/vial, white cap

Hazards identification:

H318 Causes serious eye damage.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H332 Harmful if inhaled.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.

BA E-2458 Q-BUFFER **Q-Buffer** - Ready to use

Contents: TRIS buffer

Volume: 1 x 20 ml/vial, white cap

BA E-2460 DILUENT **Diluent** - Ready to use

Contents: Buffer with sodium acetate

Volume: 1 x 20 ml/vial, dark green cap

BA E-2787	NAOH	**NaOH** - Ready to use
Contents:	Sodium hydroxide solution	
Volume:	1 x 2 ml/vial, purple cap	
Hazards identification:		

H314 Causes severe skin burns and eye damage.

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 100 µl; 12.5 ml
- Polystyrene tubes and suitable rack
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Vortex mixer
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Plasma
Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™ for plasma) and centrifuged at room temperature immediately after collection.
Fasting specimens or pre-feed specimens for children (2 - 3 hours after last meal) are advised.
Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 24 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided.

Serum
Collect blood by venipuncture ((Monovette™ or Vacuette™ for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Fasting specimens or pre-feed specimens for children (2 - 3 hours after last meal) are advised.
Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 24 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.
If 24-hour urine is used please record the total volume of the collected urine. If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence interfering factors are not extracted quantitatively.
Storage: for longer periods (up to 6 month) 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. Duplicate determinations are recommended.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.
Storage: 1 month at 2 – 8 °C

Equalizing Reagent
Reconstitute the Equalizing Reagent with **12.5 ml** of **Assay Buffer**.
Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max 1 month at -20 °C and may be thawed only once.

D-Reagent
The D-Reagent has a freezing point of 18.5 °C. To ensure that the D-Reagent is liquid when being used, it must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.

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:

- **Urine samples** with creatinine >200 mg/dl should be diluted 1:3 [e.g. 100 µl urine + 200 µl water (deionized, distilled, or ultra-pure)] before starting the Extraction step. **The results of the diluted urine samples have to be corrected for the dilution factor.**
- **Serum/plasma samples** should always be pre-diluted 1:5 [e.g. 100 µl serum/ plasma + 400 µl water (deionized, distilled, or ultra-pure)]. Serum values of Glutamate are higher than for urine. The pre-dilution step makes sure that the sample is measured in the linear range of the standard curve. **The results have to be corrected for the dilution factor**.
- 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** and this **prediluted sample** has to be used for the extraction procedure (please refer to point 6.3 of this protocol). This sample predilution has to be considered in the calculation of results (please refer to point 7 of this protocol).

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

6.3 Extraction

1.	Pipette **100 µl** of the **standards, controls** and **samples (serum 1:5 diluted)** into the appropriate wells of the **Extraction Plate.**
2.	Add **100 µl** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** 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 **samples** into the appropriate wells of the **Reaction 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 **Adhesive Foil** 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 samples** into the appropriate wells of the **Glutamate Microtiter Strips.**
2.	Pipette **50 µl** of the **Glutamate Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 h** (overnight) at **2 – 8 °C**. ***Alternatively** incubate **2 h** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).*
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 **Enzyme 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 Solution** 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

		Glutamate
Measuring range	Plasma/Serum	0.06 - 12 µg/ml
	Urine (undiluted)	0.3 – 60 µg/ml

The standard curve 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). Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

Serum/plasma
The read concentrations of **plasma samples** have to be **multiplied by 5.**

Urine samples and controls
The concentrations of the samples and controls can be read directly from the standard curve.
Diluted urine samples (refer to 6.2) have to be **multiplied by 3.**

The total amount of Glutamate excreted in urine during 24 h is calculated as following:
µg/24h = µg/ml x ml/24h

Conversion
Glutamate (µg/ml) x 6.8 = Glutamate (µmol/l)

Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

In a study conducted with apparently normal healthy adults, using the Glutamate ELISA the following values are observed:

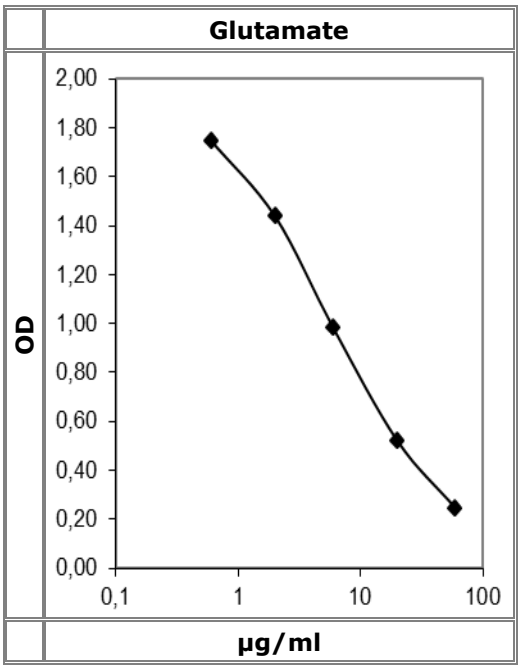
Plasma / Serum	**Spontaneous urine**
2.2 - 30 µg/ml	0.7 – 14.7 µg/g creatinine

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 commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**Glutamate**
	0.3 µg/ml

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
	Glutamate	100
	Glutamine	< 0.01
	Aspartate	0.09
	Glycine	< 0.01
	Alanine	< 0.01
	5-aminovaleric acid	< 0.01

Precision					
Intra-Assay			**Inter-Assay**		
Sample	Range (µg/ml)	CV (%)	Sample	Range (µg/ml)	CV (%)
1 (n = 25)	6.9 ± 0.5	7.3	1 (n = 19)	0.8 ± 0.18	19
2 (n = 25)	16.0 ± 1.0	6.3	2 (n = 19)	9 ± 1.2	13

Linearity		Range	Serial dilution up to	Range (%)
	Glutamate (urine)	0.61 – 47 µg/ml	1:64	83 - 111
	Glutamate (serum)	1 – 24 µg/ml	1:20	82 - 98

Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Glutamate (urine)	98	83 - 105	
	Glutamate (serum)	99	96 - 104	

9. References/Literature

(1) Pérez-Mato et al. Human recombinant glutamate oxaloacetate transaminase 1 (GOT1) supplemented with oxaloacetate induces a protective effect after cerebral ischemia. Cell Death and Disease, 5:e992 (2014)

(2) Campos et al. Glutamate oxaloacetate transaminase: A new key in the dysregulation of glutamate in migraine patients. Cephalalgia, 33(14):1148–1154 (2013)

(3) Yuan et al. Subsecond Absolute Quantitation of Amine Metabolites Using Isobaric Tags for Discovery of Pathway Activation in Mammalian Cells. Analytical Chemistry, 84(6): 2892-2899 (2012

⚠ ***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	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!