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Instructions for use

Glutamate ELISA

REF

BA E-2400

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96



RUO

For Research use only-
Not for use in diagnostic
procedures

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) The principles of Good Laboratory Practice (GLP) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) 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.
- (6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (7) 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.
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (9) 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.
- (10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (12) A standard curve must be established for each run.
- (13) 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.
- (14) 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.
- (15) 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.
- (16) 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.
- (17) 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.
- (18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (19) 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	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

Measuring range	Glutamate	
	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).



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.

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:

$$\mu\text{g}/24\text{h} = \mu\text{g}/\text{ml} \times \text{ml}/24\text{h}$$

Conversion

$$\text{Glutamate } (\mu\text{g}/\text{ml}) \times 6.8 = \text{Glutamate } (\mu\text{mol}/\text{l})$$

Expected reference values

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

Plasma / Serum	Spontaneous urine
2.2 - 30 µg/ml	5 - 100 µmol/g creatinine

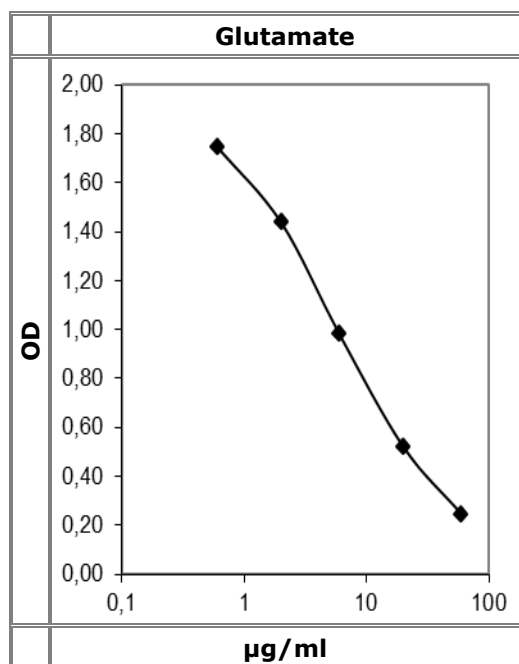
7.1 Quality control

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
	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)



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 **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	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!