

Instructions for use
Glutamate ELISA

REF**BA E-2400R**

96

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 and various biological 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 derivatized 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 research 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) The principles of Good Laboratory Practice (GLP) have to 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. Avoid repeated freezing and thawing of reagents and specimens.
- (5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (6) 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.
- (7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (8) 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.
- (9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (11) A standard curve must be established for each run.
- (12) 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.
- (13) 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.
- (14) 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.
- (15) 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.
- (16) For information on 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.
- (17) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (18) In case of any severe damage to the test kit or components, LDN has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations

2.2 Limitations

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

2.2.1 Interfering substances

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.

2.2.2 Drug 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 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	
Hazards identification:		
	H290 May be corrosive to metals.	
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	
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

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

Contents: Crosslinking agent in dimethylsulfoxide

Volume: 1 x 4 ml/vial, brown cap

Hazards
identification:

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:

H290 May be corrosive to metals.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

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
- 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

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

Urine

Urine stabilized with 10 µl 6N HCl per 1 ml of urine sample can be used.

Storage: up to 6 hours (18 – 25 °C); up to 14 days (2 – 8 °C); up to 6 months (<-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. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

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.

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.

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!

Extraction

1.	Pipette 100 µl of the standards, controls and samples 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!

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!

6.3 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 .
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
	0.26 – 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.*

Samples and Controls

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:

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

⚠ In case samples were pre-diluted correct the read values for the pre-dilution

Conversion

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

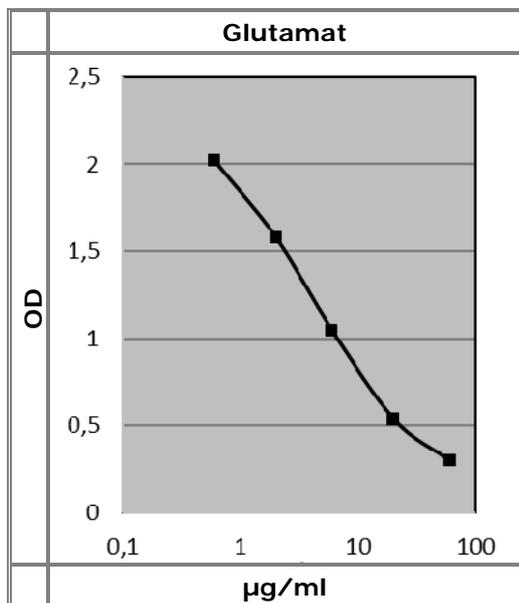
7.1 Quality control

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

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

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

Linearity		Serial dilution up to	Range (%)	Mean (%)
	Urine	1:64	94 - 113	105

Recovery		Range (µg/ml)	Range (%)	Mean (%)
	Urine	1.25 - 41.0	97 - 108	102

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

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!