Glutamate ELISA Kit

Enzyme Immunoassay for the quantification of L-Glutamate in Urine, EDTA-Plasma or Serum samples.

Catalog number: ARG80453

Package: 96 wells

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MANUFACTURED BY:

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PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative sandwich enzyme immunoassay technique. An antigen has been pre-coated onto a microtiter plate. After extraction and derivatization, controls, standards or samples are pipetted into the wells together with specific Glutamate antiserum. The standards, controls and samples and the solid phase bound analyte antigen compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing.

Following a washing to remove unbound substances, anti-rabbit IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of glutamate present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of glutamate in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Extraction Plate	2 x 48 wells (Ready-to-use)	4°C
Reaction Plate	1 X 96 wells (Ready-to-use)	4°C
Antigen-coated microplate	12 x 8 wells (Ready-to-use)	4°C
Adhesive foil	4 pieces	RT
Standard A-F (0, 0.6, 2, 6, 20, 60 µg/mL)	4 ml each (Ready-to-use)	4°C
Control 1 (1.5 µg/mL ± 40%)	4 ml (Ready-to-use)	4°C
Control 2 (4.5 µg/mL ± 40%)	4 ml (Ready-to-use)	4°C
Diluent	20 ml (Ready-to-use)	4°C
NaOH	2 ml (Ready-to-use)	4°C
Equalizing Reagent	1 vial (Lyophilized)	4°C
D-Reagent	4 ml (Ready-to-use)	4°C
Q-Buffer	20 ml (Ready-to-use)	4°C
Assay buffer	20 ml (Ready-to-use)	4°C
Glutamate antiserum	6 ml (Ready-to-use)	4°C
Anti-rabbit IgG peroxidase conjugate	12 ml (Ready-to-use)	4°C (Protect from light)
50X Wash buffer	20 ml	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620-650 nm as reference and 405nm if needed)
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- 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.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Ensure complete reconstitution and dilution of reagents prior to use.
- 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.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.

- In rare cases residues of the blocking and stabilizing reagent can be seen in the wells of Glutamate Microtiter Strips as small, white dots or lines. These residues do not influence the quality of the product.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C up to 6 month. Avoid repeated freeze-thaw cycles.

Note:

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.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C up to 6 month. Avoid repeated freeze-thaw cycles.

Note:

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

Urine – Collect urine with 10µl of 6M HCl per 1ml of urine, and use it immediately or within 6 hours is recommended. The sample is stable for up to 6 hours at room temperature, and for up to 14 days when stored at 4°C. For a longer period storage, please store at ≤-20 °C for up to 6 months. Avoid exposure to direct sunlight. Repeated freeze-thawing is not advisable.

Note:

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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. E.g. dilute the 20 ml of 50X Wash Buffer into distilled water to a final volume of 1000 ml. The diluted wash buffer can be stored at 4°C for 1 month.
- Equalizing Reagent: Reconstitute the lyophilized Equalizing Reagent with 12.5 ml of Assay Buffer. Unused reconstituted equalizing reagent need to be stored in aliquots at-20 °C up to 1 month 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.

SAMPLE PREPARATION

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**, **distilled water** has to be added to a **final volume of 100 µl** and this pre-diluted sample has to be used for the extraction procedure (please refer to next section). This sample pre-dilution has to be considered in the calculation of results.

• If the initial assay found samples contain Glutamate higher than the highest standard, the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

(It is recommended to do pre-test to determine the suitable dilution factor).

ASSAY PROCEDURE

- Extraction

1. Add **100 µl** of **standards, controls** and **samples** into the **Extraction Plate**.
2. Add **100 µl** of the **Diluent** into each well. Cover the plate with adhesive foil and incubate for **10 minutes at RT** on a microplate shaker (600 rpm).
3. Use **25 µl** for the subsequent derivatization procedure.

- Derivatization

1. Add **25 µl** of **extracted standards, controls** and **samples** in duplicate into the **Reaction Plate**.
2. Add **10 µl** of **NaOH** into all well.
3. Add **50 µl** of the **Equalizing Reagent** into all wells.
4. Add **10 µl** of the **D-Reagent** into all wells.
5. Cover the wells with adhesive foil and incubate for **2 hours at RT** on a microplate shaker at 600rpm.
6. Add **75 µl** of the **Q-Buffer** into all wells. Shake at 600 rpm on a microplate shaker for **10 min at RT**.
7. Use **25 µl** for the ELISA assay.

- Glutamate ELISA Procedure

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame (Antigen-coated microplate), return them to the foil pouch containing the desiccant pack, and reseal it.

2. Add **25 µl** of the **derivatized standards, controls** and **samples** in duplicates into the Glutamate-coated microtiter strips (Antigen-coated microplate).
3. Add **50 µl** of the **Glutamate antiserum** into each well, mix shortly.
4. Cover the wells with adhesive foil and incubate for **15-20 hours at 2-8°C**
5. Remove the foil and discard. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of the **Anti-rabbit IgG peroxidase conjugate** into each well. Incubate on a microplate shaker (600 rpm) for **30 mins at RT.**
7. Aspirate each well and **wash as step 5**.
8. Add **100 µl** of **TMB Substrate** to each well. Incubate on a microplate shaker (600 rpm) for **20-30 mins at RT** in dark.
9. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at **450 nm** (with a reference wavelength between 620nm and 650nm) within 10 minutes.

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

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The concentrations of undiluted samples and controls can be read directly from the standard curve.

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

7. For topped-up samples, the concentration has to be multiplied with a volume factor:

Volume Factor = 100 µl/used sample volume (µl)

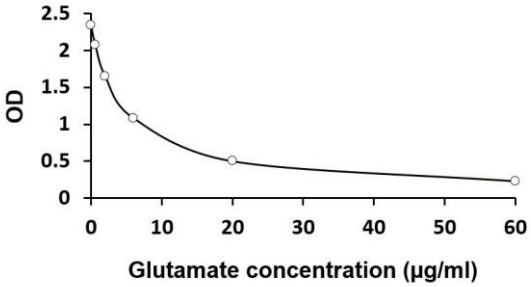
8. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

9. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Glutamate (µg/ml)	0	0.6	2	6	20	60
Glutamate (µmol/L)	0	4.08	13.6	40.8	136	408
Conversion	Glutamate (µg/ml) x 6.8 = Glutamate (µmol/L)					

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time



QUALITY ASSURANCE

Sensitivity

The standard of Glutamate ranged from 0-60 µg/ml.

Limit of Blank (LOB) was 0.11 µg/ml.

Limit of Detection (LOD) was 0.17µg/ml.

Limit of Quantification (LOQ) was 0.26 µg/ml.

Specificity

	Substance	Cross Reactivity (%)
Analytical Specificity	Glutamate	100
	L-Glutamine	< 0.4
	L-Aspartic Acid	< 0.4
	Glycine	< 0.4
	L-Alanine	< 0.4
	5-Amino-n-valeric Acid	< 0.4
	GABA	< 0.4

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.67% and inter-assay precision was 11.3%.

Linearity

Urine: 94- 113%

Recovery

Urine: 97- 108%