Glutamate ELISA Kit

Catalog Number KA1909

96 assays

Version: 07

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of L-Glutamate in urine and various biological samples.

Principle of the Assay

After extraction and derivatization 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 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.

General Information

Materials Supplied

List of component

Component	Detail	Amount
Adhesive Foil	Ready to use, in a resealable pouch.	4 slides
Reaction Plate	Ready to use, empty in a resealable pouch.	96 well plate x 1
Extraction Plate	Ready to use, precoated with cation exchanger in a resealable pouch.	48 well plate x 2
Wash Buffer Concentrate (50x)	Concentrated buffer with a non-ionic detergent and physiological pH.	20 mL
Enzyme Conjugate	Ready to use, goat anti-rabbit immunoglobulins conjugated with peroxidase.	12 mL
Substrate	Ready to use, chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide.	12 mL
Stop Solution	Ready to use, containing 0.25 M sulfuric acid. Hazards identification:  H290 May be corrosive to metals.	12 mL
Glutamate Microtiter Strips	Ready to use, antigen precoated microwell plate in a resealable foil pouch with desiccant.	96 (8x12) wells
Glutamate Antiserum	Ready to use, rabbit anti-glutamate antibody, blue coloured.	6 mL
Assay Buffer	Ready to use, buffer with alkaline pH.	20 mL
Equalizing Reagent	Lyophilized protein.	1 vial
D-Reagent	Ready to use, crosslinking agent in dimethylsulfoxide. Hazards identification:  H317 May cause an allergic skin reaction.	4 mL
Q-Buffer	Ready to use, TRIS buffer.	20 mL
Diluent	Ready to use, buffer with sodium acetate.	20 mL
NaOH	Ready to use, sodium hydroxide solution. Hazards identification:  H290 May be corrosive to metals. H315 Causes skin irritation. H319 Causes serious eye irritation.	2 mL

Standards and Controls - Ready to use

Component	Concentration (µg/mL)	Concentration (µmol/L)	Amount
Standard A	0	0	4 mL
Standard B	0.6	4.08	4 mL
Standard C	2	13.6	4 mL
Standard D	6	40.8	4 mL
Standard E	20	136	4 mL
Standard F	60	408	4 mL
Control 1	Refer to QC-Report for expected value and acceptable range.		4 mL
Control 2	Refer to QC-Report for expected value and acceptable range.		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.

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ 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)

Precautions for Use

- ✓ 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.
- ✓ The principles of Good Laboratory Practice (GLP) have to be followed.
- ✓ In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- ✓ 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.
- ✓ For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- ✓ 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.
- ✓ Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- ✓ 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.
- ✓ Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- ✓ To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- ✓ A standard curve must be established for each run.
- ✓ 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.
- ✓ 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.
- ✓ 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.
- ✓ 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.
- ✓ 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.
- ✓ Kit reagents must be regarded as hazardous waste and disposed according to national regulations.
- ✓ In case of any severe damage to the test kit or components, we have 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 office regulations.

- Limitations

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

  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.
- Drug interferences

  There are no known substances (drugs, food) which ingestion interferes with the measurement of glutamate level in the sample.
- High-Dose-Hook effect

  No hook effect was observed in this test.

Assay Protocol

Reagent Preparation

- 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.

Sample Preparation

✓ Sample collection and storage
Various biological samples can be used for L-Glutamate determination. The assay was validated for urine sample.

- Urine
  Urine stabilized with 10 µL 6 N 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.

✓ Preparation of samples
The Glutamate ELISA Kit 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.

Assay Procedure

Allow all reagent 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 conjugates 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.
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.

✓ 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 hours 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!

✓ 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 hours (overnight) at 2 – 8°C.

4. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3x 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 3x 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). *Note: 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).

Data Analysis

Calculation of Results

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).
Note: 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.

- ✓ Measuring range: 0.26-60 µg/mL
- ✓ 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 correction factor:

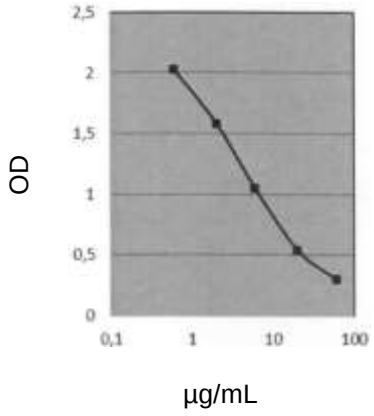
  $$\text{Correction factor} = \frac{\text{100 µL (volume of standards)}}{\text{sample volume (µL)}}$$

  In case samples were pre-diluted correct the read values for the pre-dilution.
- ✓ Conversion

  Glutamate (µg/mL) x 6.8 = Glutamate (µmol/L)
- ✓ Quality control

  The confidence limits of the kit controls are indicated on the QC-Report.
- ✓ Typical standard curve

  Example, do not use for calculation!



Performance Characteristics

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

- Analytic Sensitivity

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	10.1 ± 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

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H