ab83389

Glutamate Assay kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Glutamate in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 8c, Last Updated 12 August 2025

Table of Contents

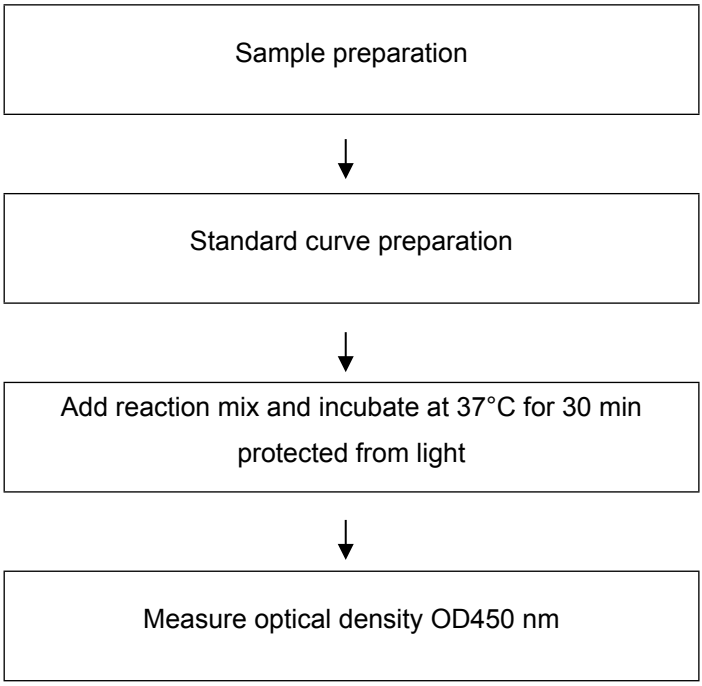
1. BACKGROUND

Glutamate Assay Kit (Colorimetric) (ab83389) provides a sensitive detection method of the glutamate in a variety of samples. This kit will only measure free glutamate levels but not glutamic acid found in the backbone of peptides or proteins. The Enzyme Mix VIII recognizes glutamate as a specific substrate leading to proportional color development. The amount of glutamate can therefore be easily quantified by colorimetric (spectrophotometry at OD = 450 nm) method.

Glutamate, one of the two acidic proteinogenic amino acids, is also a key molecule in cellular metabolism. In humans, glutamate plays an important role both in amino acid degradation and disposal of excess or waste nitrogen. Glutamate is the most abundant swift excitatory neurotransmitter in the mammalian nervous system. It is believed to be involved in learning and memory and has appeared to be involved in diseases like amyotrophic lateral sclerosis, lathyrism, autism, some forms of mental retardation and Alzheimer's disease. Glutamic acid is also present in a wide variety of foods, and has been used as a flavour enhancer in food industry.

PLEASE NOTE: EDTA plasma may not be used with the ab83389 Glutamate Assay Kit. EDTA can interfere with the assay.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 31	25 mL	-20°C	-20°C
Enzyme Mix VIII	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	-20°C
Glutamate Standard	0.1 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 31 was previously labelled as Assay Buffer XXXI and Glutamate Assay Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD450 nm
- 96 well plate: clear plates for colorimetric assay
- Orbital shaker
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 31:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Enzyme Mix VIII:**

Reconstitute Glutamate Enzyme Mix VIII with 220 µL Assay Buffer 31. Aliquot Enzyme Mix VIII so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Use within two months. Do not freeze-thaw more than 5 times. Keep on ice during the assay and protected from light.

9.3 **Developer Solution III:**

Reconstitute Developer Solution III with 820 µL of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution, do not vortex. Aliquot Developer Solution III so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice during the assay.

9.4 **Glutamate Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice during the assay.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 1 mM Glutamate standard by diluting 5 µL of the 0.1 M Glutamate Standard in 495 µL of Assay Buffer 31.

10.2 Using 1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 31 (µL)	Final volume standard in well (µL)	End [glutamate] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples):

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL Assay Buffer 31.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Incubate cells for 15 – 30 min on ice.

11.1.6 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.1.8 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Assay Buffer 31.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Incubate sample for 15 – 30 min on ice.

11.2.6 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.7 Collect supernatant and transfer to a clean tube.

11.2.8 Keep on ice.

11.3 **Serum and urine samples:**

Serum samples can be tested directly by adding sample to the microplate wells, but urine samples should not be tested directly. Bring volumes up to 50 µL with Assay Buffer 31.

The urea in urine can cause a high background. Therefore, it is important to deproteinize the samples and use activated charcoal to reduce the colour. It is recommended to maintain the sample volume below 20 µL/well. PCA/KOH should be used for deproteinization. Deproteinizing-TCA kit (ab204708) is not recommended for this product.

To find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

11.4 **Food samples:**

For liquid food samples (e.g., soya sauce), we recommend performing several dilutions of the sample. (1/50 – 1/100 – 1/250 – 1/500).

11.4.1 For solid food samples (e.g., tomato): chopped in small pieces (you can use a food blender to improve extraction of glutamate) and homogenize in Assay Buffer 31 (1 g sample/mL assay buffer).

11.4.2 Incubate homogenate for a minimum of 10 minutes at room temperature.

11.4.3 Centrifuge samples for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove insoluble material.

11.4.4 Collect supernatant and transfer to a clean tube.

Recommended dilutions: 1/25 – 1/50 – 1/100 – 1/150

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL Standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 31).
- Background control sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 31).

12.2 **Reaction Mix:**

Prepare 100 µL of Reaction Mix for each reaction:

Component	Reaction Mix Samples (µL)	Background Reaction Mix (µL)
Assay Buffer 31	**90**	**92**
Developer Solution III	**8**	**8**
Enzyme Mix VIII	**2**	**0**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1).

12.3 Add 100 µL of Reaction Mix into each standard and sample wells.

12.4 Add 100 µL of Background Reaction Mix into Background sample wells.

12.5 Mix and incubate at 37°C for 30 minutes protected from light.

12.6 Measure output (OD450 nm) on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 If the sample background control is significant, then subtract the sample background control from sample readings.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Glutamate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Sa = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of samples in the test samples is calculated as:

$$Glutamate\ Concentration = \left(\frac{Sa}{Sv}\right) * D$$

Where:

Sa = Amount of sample (nmol) from standard curve.

Sv = Volume of sample (µL) added into the well.

D = Sample dilution factor.

L-Glutamic acid Molecular Weight is 147.13g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

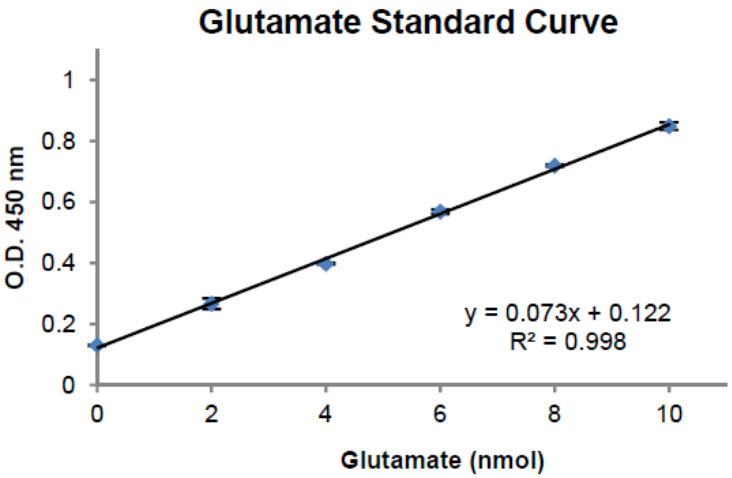


Figure 1: Typical Glutamate standard calibration curve using colorimetric reading.

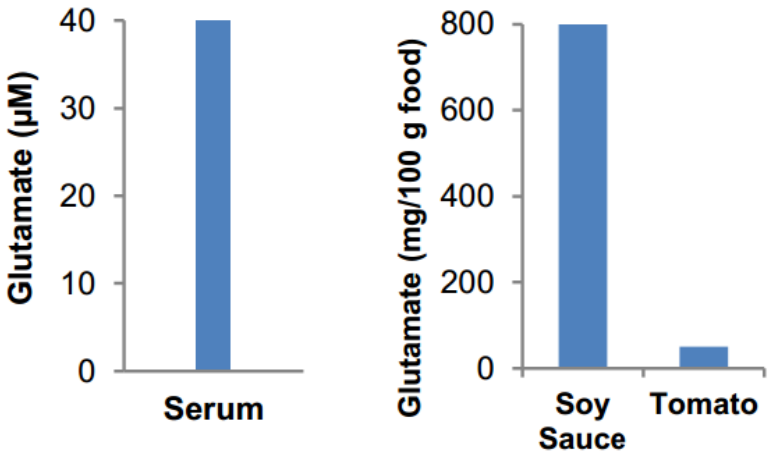


Figure 2: Glutamate concentration in various samples. Left graph shows concentration of glutamate in human serum (25 µL). Right graph shows concentration of glutamate in soy sauce (25 µL, 500X diluted) and fresh tomato (25 µL, 50X diluted). Enzyme Mix VIII is inhibited by tomato homogenate. Assays were performed following the kit protocol.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Solubilize Enzyme Mix VIII and Developer Solution III, thaw Glutamate Standard and Assay Buffer 31 (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background sample wells (50 µL).
- Prepare Glutamate Reaction Mix and Background Reaction Mix (number samples + standards + 1).

Component	Reaction Mix Samples (µL)	Background Reaction Mix (µL)
Assay Buffer 31	**90**	**92**
Developer Solution III	**8**	**8**
Enzyme Mix VIII	**2**	**0**

- Add 100 µL of Reaction Mix to standard and sample wells.
- Add 100 µL Background Reaction Mix to background sample wells.
- Incubate plate at 37°C for 30 min protected from light.
- Measure plate at OD450 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQs

Does this assay only measure free glutamate or can it also measure the amount of glutamic acid in peptides/proteins?

This kit measures free glutamate levels only and not glutamic acid in the backbone of peptides/proteins.

Does glutamine cross-react during this assay?

This assay uses enzymatic detection which reacts with glutamate and not glutamine.

What is the detection sensitivity of the assay?

The kit shows a linear standard curve up to 200 µM Glutamate.

Why there is an increase in the glutamate level in astrocytes cell culture?

Astrocytes release glutamate in a Ca^{2+}-dependent manner. It is a well-studied function required during transmitter uptake and release. The rate of release can be plotted against time and then the time when the release is minimum it can be for intracellular glutamate assay. Otherwise, total glutamate in medium + cells can be measured.

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

19. **NOTES**

Technical Support

Copyright © 2025 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)