Glutamate Assay Kit
Catalogue Number: OM700065
(96 Tests)
Store all reagents at -20°C Dark
Collect sample: serum or blood plasma
Assay range ： 2-10nmol

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

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.

PRINCIPLE OF THE ASSAY

Glutamate Assay Kit is a quantitative, addition-only assay with just one 30-min incubation step. Readout on any colorimetric (450 nm) plate reader. Use as a

free glutamate assay (does not detect glutamic acid incorporated in peptides or proteins) -Cited in over 80 publications -Individual kit components also available for purchase with a minimum order of 20 units. Contact us to discuss your needs.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1.Assay Buffer 31	25 mL 1 tube
2. Enzyme Mix VIII	1 vial
3.Developer Solution III	1 vial
4. Glutamate Standard	0.1 mL 1 tube
5. Instruction	1 copy

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.

Cell (adherent or suspension) samples

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

Wash cells with cold PBS.

Resuspend cells in 100 µL Assay Buffer 31.

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

Incubate cells for 15 – 30 min on ice.

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

Collect supernatant and transfer to a clean tube.

Keep on ice.

Tissue samples

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

Wash tissue in cold PBS.

Resuspend tissue in 100 µL of Assay Buffer 31.

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

Incubate sample for 15 – 30 min on ice.

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

Collect supernatant and transfer to a clean tube.

Keep on ice.

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 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).

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).

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).

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

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

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.

MATERIALS REQUIRED BUT 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 (ddH2O) □
- 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)

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.

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.

REAGENT PREPARATION

Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer 31

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

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℃ 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.

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℃. Keep on ice during the assay.

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℃. Keep on ice during the assay.

STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Prepare a 1 mM Glutamate standard by diluting 5 µL of the 0.1 M Glutamate Standard in 495 µL of Assay Buffer 31.
- 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).

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.

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).

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).

- Add 100 µL of Reaction Mix into each standard and sample wells.
- Add 100 µL of Background Reaction Mix into Background sample wells.
- Mix and incubate at 37° C for 30 minutes protected from light. 12.6 Measure output (OD450 nm) on a microplate reader.

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).
  Average the duplicate reading for each standard and sample.
  Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
  If the sample background control is significant, then subtract the sample background control from sample readings.
  Plot the corrected absorbance values for each standard as a function of the final concentration of Glutamate.
  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).
  Extrapolate sample readings from the standard curve plotted using the following equation:

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

  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.

TYPICAL DATA

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

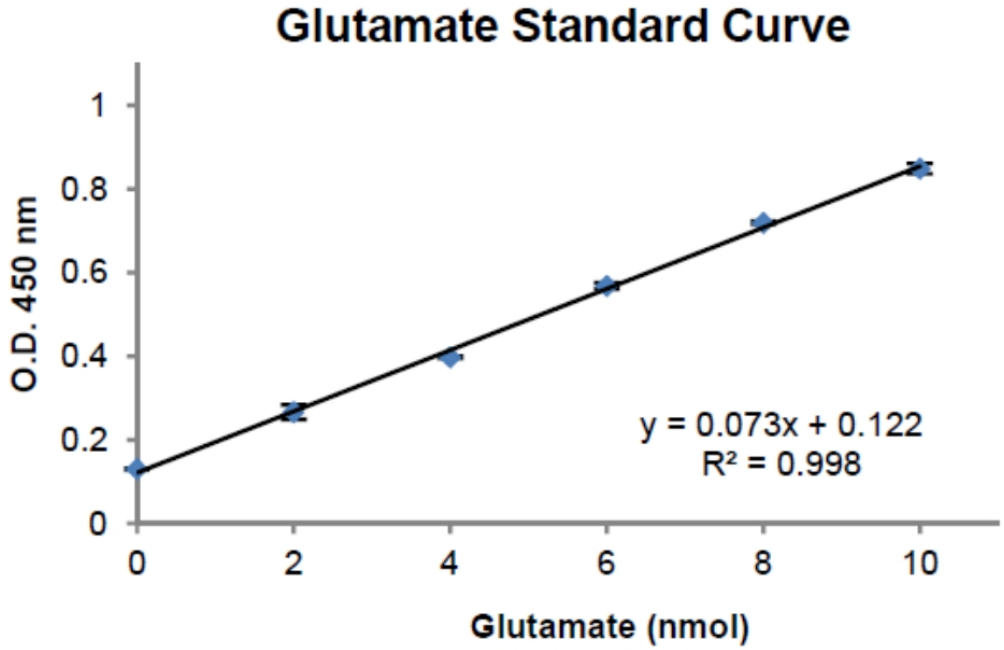


Figure 1: Typical Glutamate standard calibration curve using colorimetric reading.

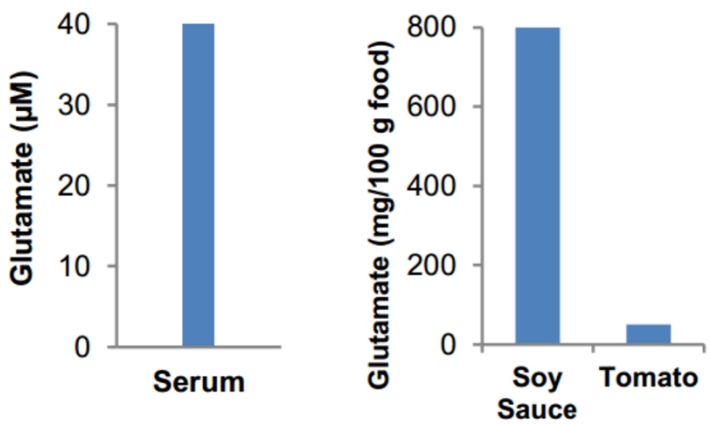


Figure 2: Glutamate concentration in various samples. Left graph shows concentration of glutamate in human serum (25 µL). Right graph shows concentration of glutamate in soy sauce (25 µL, 500X diluted) and fresh tomato (25 µL, 50X diluted). Enzyme Mix VIII is inhibited by tomato homogenate. Assays were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