Product Manual

Glutamate Assay Kit (Fluorometric)

Catalog Number

STA-674 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

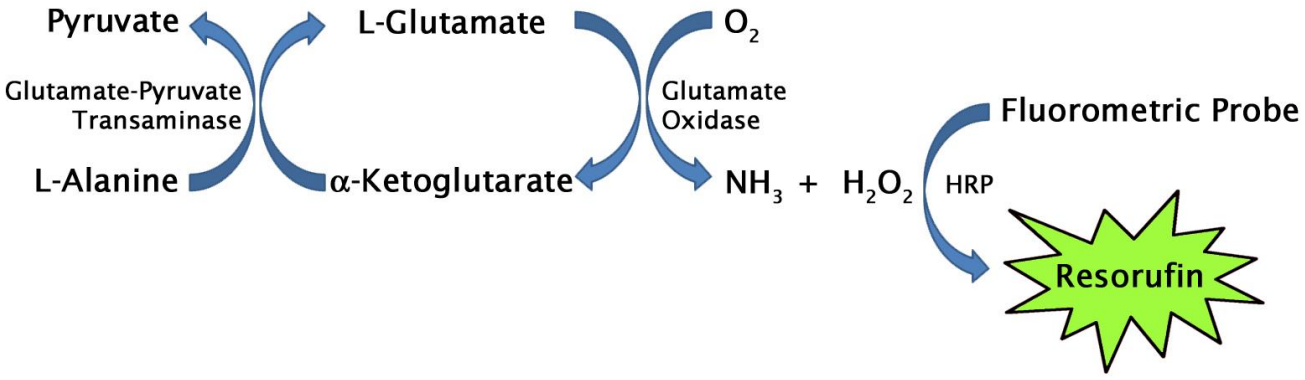
Introduction

Glutamate is a non-essential amino acid that has a key metabolic role in processes such as the citric acid cycle and removal of excess nitrogen waste. In its monosodium form (MSG), glutamate is well known as a flavor enhancer. Glutamate has also been identified as one of the major excitatory neurotransmitters of the mammalian brain. Glutamate is involved in learning and memory, and long-term potentiation occurs at glutaminergic synapses. In addition, glutamate helps to regulate growth cones and synaptogenesis. Postsynaptically, glutamate has been suggested to activate the NMDA, AMPA, and kainite receptors. Damage and/or death to nerve cells due to excessive glutamate release and deficits in uptake have been correlated with diseases such as amyotrophic lateral sclerosis, lathyrism, and Alzheimer's disease as well as stroke, autism, and some forms of intellectual disability.

Cell Biolabs' Glutamate Assay Kit is a simple HTS-compatible assay for measuring glutamate levels in biological samples without any need for pretreatment. The assay uses glutamate-specific enzymes to generate H_2O_2. In the presence of H_2O_2 and horseradish peroxidase (HRP), the non-fluorescent Fluorometric Probe is oxidized to the highly fluorescent Resorufin. The kit has a detection sensitivity limit of 300 nM glutamate. Each kit provides sufficient reagents to perform up to 200 assays, including standard curve and unknown samples.

Assay Principle

The Glutamate Assay Kit is a sensitive quantitative fluorometric assay for glutamate. Glutamate oxidase converts glutamate to α-ketoglutarate and also produces NH_3 as well as H_2O_2. L-alanine and glutamate-pyruvate transaminase are also added to the reaction in order to regenerate glutamate. As a result, multiple rounds of the reaction occur which results in significant amplification of H_2O_2 production. In the presence of HRP, the Fluorometric Probe reacts with H_2O_2 in a 1:1 stoichiometry to produce highly fluorescent Resorufin. The Resorufin product can be easily read by a fluorescence microplate reader with an excitation of 530-560 nm and an emission of 590 nm. Fluorescence values are proportional to the glutamate levels within the samples. The glutamate content in unknown samples is determined by comparison with a standard curve.



Related Products

1. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
2. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
3. STA-341: OxiSelect™ Catalase Activity Assay Kit
4. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit

Kit Components (shipped on blue ice)

1. Glutamate Oxidase (Part No. 267402): One 160 µL vial at 5 U/mL.

   Note: One unit is defined as the amount of enzyme that will form 1.0 micromole of alpha-ketoglutaric acid from L-glutamic acid per minute at pH 7.4 at 30°C.

2. Glutamate-Pyruvate Transaminase (Part No. 267403): One 50 µL vial at 100 U/mL.

   Note: One unit is defined as the amount of enzyme that will cause the transamination of 1.0 µmole of L-alanine per minute at pH 7.5 and 25°C.

3. L-Alanine (Part No. 267404): One 10 µL vial at 200 mM.
4. L-Glutamate Standard (Part No. 267405): One 100 µL vial at 20 mM.
5. Fluorometric Probe (Part No. 268101): One 250 µL amber tube of a 10 mM solution in DMSO.
6. HRP (Part No. 234402): One 100 µL tube.
7. 10X Assay Buffer (Part No. 267401): One 25 mL bottle of 1 M Tris pH 7.4.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS for sample dilutions
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate
6. Multichannel micropipette reservoir
7. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Aliquot and store all other components at -20ºC. Avoid multiple freeze/thaw cycles. The Fluorometric Probe is light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by adding the Fluorometric Probe to a final concentration of 100 µM, HRP to a final concentration of 0.2 U/mL, Glutamate Oxidase to 0.08 U/mL, Glutamate-Pyruvate Transaminase to 0.5 U/mL, and L-Alanine to 200 µM in 1X Assay Buffer. For example, add 50 µL Fluorometric Probe stock solution, 10 µL HRP stock solution, 80 µL of Glutamate Oxidase, 25 µL of Glutamate-Pyruvate-Transaminase, and 5 µL of L-Alanine to 4.83 mL 1X Assay Buffer for a total of 5 mL. This Reaction Mix volume is enough for ~100 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Scale down the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Glutamate standard curve in the same non-conditioned media.

  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

  Notes:
  - *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
  - *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
  - *Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Glutamate standards before use by diluting in 1X Assay Buffer. First, dilute the stock L-Glutamate Standard 20 mM solution 1:10 in 1X Assay Buffer for a 2 mM Glutamate Solution. (e.g. add 5 µL of the stock 20 mM L-Glutamate Standard to 45 µL of 1X Assay Buffer). Use the 2 mM Glutamate Solution to prepare a series of the remaining Glutamate standards according to Table 1 below.

Standard Tubes	2 mM Glutamate Solution (µL)	1X Assay Buffer (µL)	Glutamate (µM)
1	5	495	20
2	250 of Tube #1	250	10
3	250 of Tube #2	250	5
4	250 of Tube #3	250	2.5
5	250 of Tube #4	250	1.25
6	250 of Tube #5	250	0.625
7	250 of Tube #6	250	0.3125
8	0	250	0

Table 1. Preparation of Glutamate Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each sample (Glutamate standard or unknown) into wells of a fluorescence black microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30-45 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of glutamate within samples by comparing the sample RFU to the standard curve.

Example of Results

The following figures demonstrate typical Glutamate Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

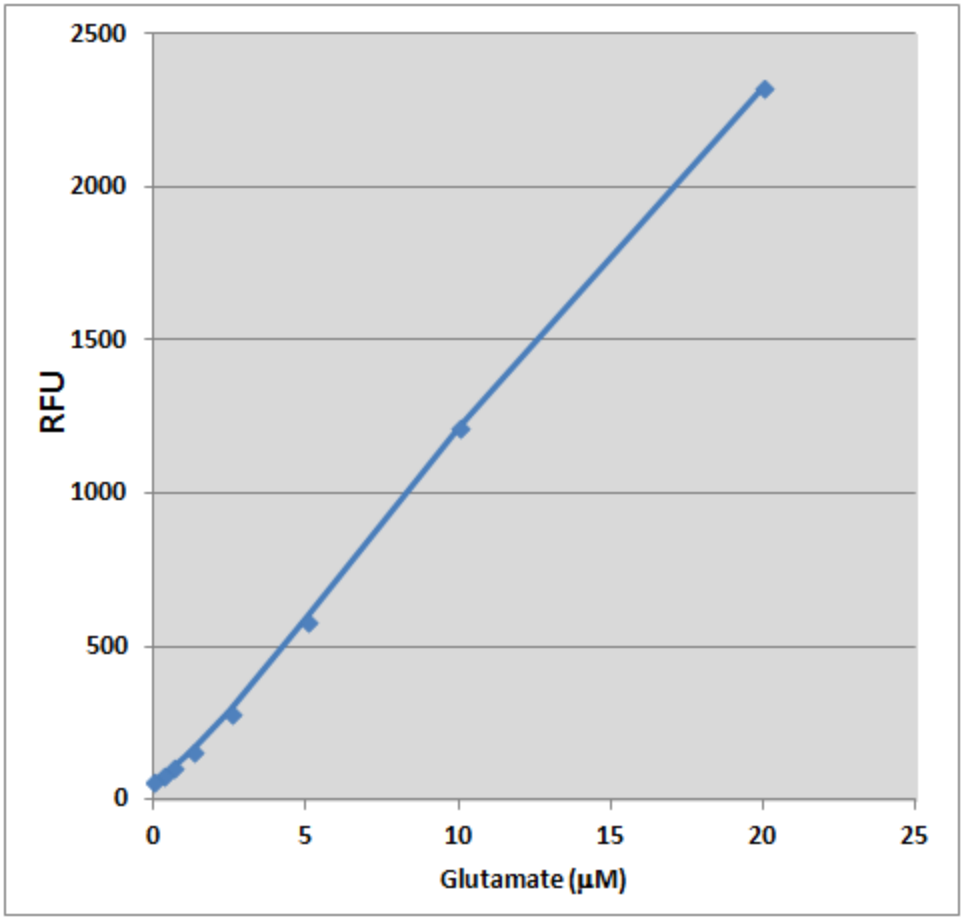


Figure 1. Glutamate Standard Curve.

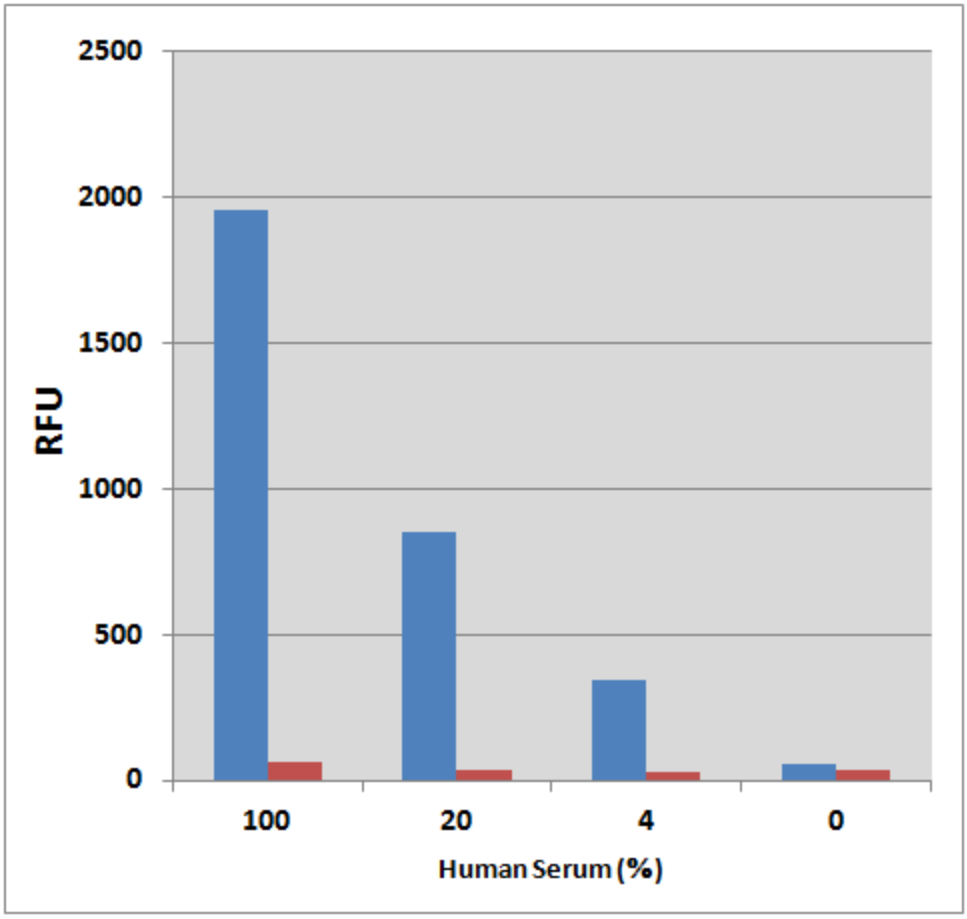


Figure 2. Detection of glutamate in human serum. 50 µL of pooled human serum was incubated with Fluorometric Probe, HRP, and Alanine in the presence (blue bars) or absence (red bars) of Glutamate Oxidase and Glutamate-Pyruvate Transaminase.

References

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Recent Product Citations

1. Lange, J. et al. (2023). PolyQ length-dependent metabolic alterations and DNA damage drive human astrocyte dysfunction in Huntington's disease. *Prog Neurobiol*. **225**:102448. doi: 10.1016/j.pneurobio.2023.102448.

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5. Haider, S. et al. (2018). Impact of 1-day and 4-day MWM training techniques on oxidative and neurochemical profile in rat brain: A comparative study on learning and memory functions. *Neurobiol Learn Mem*. **155**:390-402. doi: 10.1016/j.nlm.2018.09.003.

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