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Datasheet for ABIN5067613

Glutamate Assay Kit

3 Images

Overview

Quantity:	200 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The Glutamate Assay Kit is a sensitive quantitative fluorometric assay for glutamate.
Sample Type:	Urine, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	<p>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 H₂O₂. In the presence of H₂O₂ 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.</p>
Components:	<ol style="list-style-type: none">1. Glutamate Oxidase : 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 : 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 : One 10 μL vial at 200 mM.4. L-Glutamate Standard : One 100 μL vial at 20 mM.5. Fluorometric Probe : One 250 μL amber tube of a 10 mM solution in DMSO.6. HRP : One 100 μL tube.

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7. 10X Assay Buffer : One 25 mL bottle of 1 M Tris pH 7.4.

Target Details

Background: 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.

Application Details

Application Notes: Optimal working dilution should be determined by the investigator.

Comment:

- Detection sensitivity limit of 0.3 μ M glutamate
- Suitable for use with lysates, cell culture supernatants, serum, plasma, and urine

Protocol: 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.

Reagent Preparation: 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 μ M, 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 μ M in 1X Assay Buffer. For example, add 50 μ L Fluorometric Probe stock solution, 10 μ L HRP stock

Application Details

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 4 °C. Note: Scale down the described example appropriately and prepare only enough for immediate use.

- Sample Preparation:
- 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 10⁶ 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).

- Assay Procedure:
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 37 °C 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. 5

Restrictions: For Research Use only

Handling

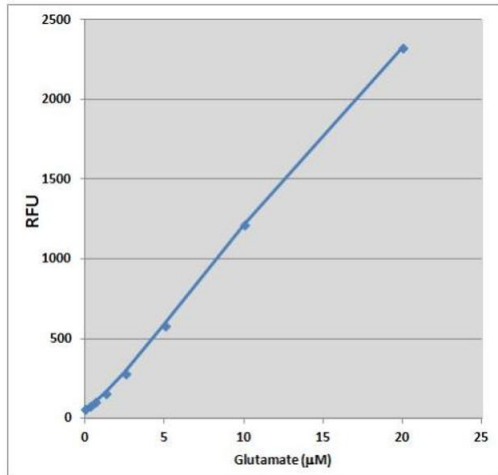
Handling Advice: Avoid multiple freeze/thaw cycles.

Storage: 4 °C/-20 °C

Handling

Storage Comment: Upon receipt, store the 10X Assay Buffer at 4°C. Aliquot and store all other components at -20°C. Avoid multiple freeze/thaw cycles. The Fluorometric Probe is light sensitive and must be stored accordingly. 3

Images



Biochemical Assay

Image 1. Glutamate Standard Curve.

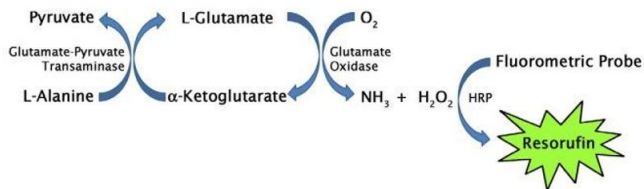
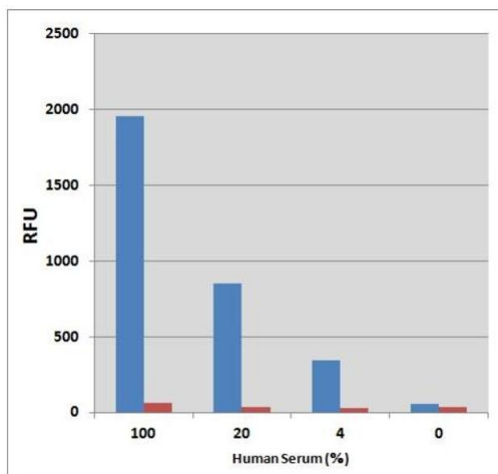


Image 2. Assay Principle



Biochemical Assay

Image 3. 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.