

Glutamate Assay Kit

(Catalog #K629-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Glutamate is 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 flavor enhancer in food industry. BioVision's Glutamate Assay Kit provides a sensitive detection method of the glutamate in a variety of samples. The glutamate Enzyme Mix recognizes glutamate as a specific substrate leading to proportional color development. The amount of glutamate can therefore be easily quantified by colorimetric (spectrophotometry at $\lambda = 450$ nm) method.

II. Kit Contents:

Components	K629-100	Cap Code	Part No.
Glutamate Assay Buffer	25 ml	WM	K629-100-1
Glutamate Enzyme Mix	1 vial	Green	K629-100-2
Glutamate Developer	1 vial	Red	K629-100-3
Glutamate Standard (0.1M)	0.1 ml	Yellow	K629-100-4

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Reconstitute Glutamate Enzyme Mix with 220 μ l Assay Buffer. Reconstitute Glutamate developer with 820 μ l of ddH₂O. Pipette up and down several times to completely dissolve the pellet into solution (**Don't vortex**). Aliquot enough Glutamate Enzyme Mix (2 μ l per assay) for the number of assays to be performed in each experiment and freeze the stock solution immediately at -20°C for future use. The Glutamate Enzyme Mix is stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycle less than 5 times.

Ensure that the Assay Buffer is at room temperature before use. Keep the Glutamate Enzyme Mix on ice during the assay and protect from light.

V. Glutamate Assay Protocol:

1. Glutamate Standard Curve:

Dilute 10 μ l of the 0.1M Glutamate standard with 990 μ l Assay Buffer to generate 1 mM standard Glutamate. Add 0, 2, 4, 6, 8, 10 μ l of the diluted Glutamate standard into a 96-well plate in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 μ l with Assay buffer.

2. Sample Preparations:

Tissues or cells (1×10^6) can be homogenized in 100 μ l Assay Buffer centrifuge to remove insoluble material at 13,000 g, 10 minutes. 10-50 μ l serum samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 μ l/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 100 μ l Reaction Mix containing:

90 μ l Assay Buffer
8 μ l Glutamate Developer
2 μ l Glutamate Enzyme Mix

Add 100 μ l of the **Reaction Mix** to each well containing the Glutamate Standard and test samples. Mix well. Incubate the reaction for 30 min at 37°C, protect from light.

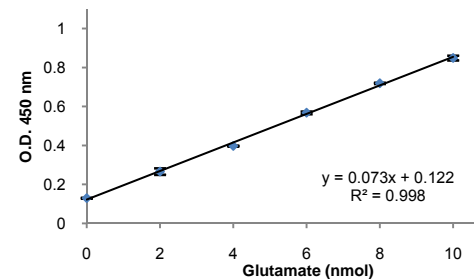
4. Measure O.D. at 450 nm in a microplate reader.

5. Correct background by subtracting the value derived from the 0 Glutamate control from all sample readings (The background reading can be significant and must be subtracted from sample readings). Plot Glutamate standard Curve, Glutamate concentrations of the test samples can then be calculated:

$$C = S_a/S_v \text{ nmol}/\mu\text{l, or mM}$$

where S_a is the sample amount of unknown (in nmol) from standard curve,
 S_v is sample volume (μ l) added into the wells.
L-Glutamic acid Molecular Weight is 147.13 g/mol.

Glutamate Standard Curve



VI. Related Products:

NAD/NADH Quantification Kit
ADP/ATP Ratio Assay Kit
Glucose Assay Kit
Ethanol Assay Kit
Pyruvate Assay Kit
Creatine Assay Kit
Ammonia Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Sarcosine Assay Kit
Nitric Oxide Assay Kit

NADP/NADPH Quantification Kit
Ascorbic Acid Quantification Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit
Lactate Assay Kit/ II
Creatinine Assay Kit
Free Glycerol Assay Kit
Hemin Assay Kit
Total Antioxidant Capacity (TAC) Assay Kit
L-amino Acid Assay Kit
Glutathione Detection Kit