# Glutamate Colorimetric Assay Kit

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

## I. Introduction:

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 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 λ = 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 µl Assay Buffer. Reconstitute Glutamate developer with 820 µl of $ddH_2O$. Pipette up and down several times to completely dissolve the pellet into solution (**Don't vortex**). Aliquot enough Glutamate Enzyme Mix (2 µl 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 µl of the 0.1M Glutamate standard with 990 µl Assay Buffer to generate 1 mM standard Glutamate. Add 0, 2, 4, 6, 8, 10 µl 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 µl with Assay buffer.

2. **Sample Preparations:**
   Tissues or cells ($1\times10^6$) can be homogenized in 100 µl Assay Buffer. Centrifuge to remove insoluble material at 13,000 g, 10 minutes. 10-50 µl serum samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 µl/well with Assay Buffer in a 96-well plate. Prepare a parallel sample well as the background control. 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 µl Mix containing:

| Reaction Mix | Background Control Mix |
|---|---|
| 90 µl Assay Buffer | 92 µl Assay Buffer |
| 8 µl Glutamate Developer | 8 µl Glutamate Developer |
| 2 µl Glutamate Enzyme Mix | |

   Add 100 µl of the Reaction Mix to each well containing the Glutamate Standard and test samples. To the background control well, add 100 µl of background control mix. Mix well. Incubate the reaction for 30 min at 37°C, protected from light.

4. Measure OD at 450 nm in a microplate reader.

5. Correct background by subtracting the value derived from background 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:

   $C = S_a/S_v$ nmol/µl, or mM

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

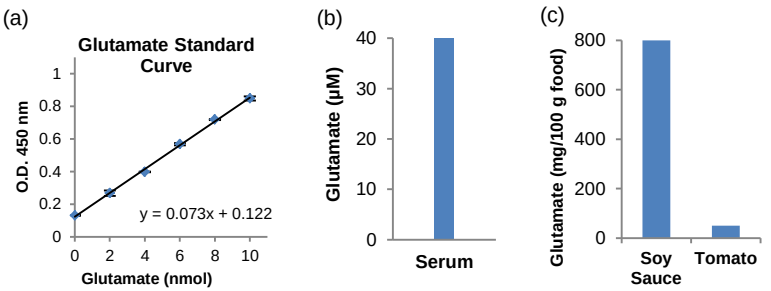


**Figure.** Glutamate Standard Curve (a). Glutamate concentration in human serum (25 µl) (b), and soy sauce (25 µl, 500 times diluted) and tomato (fresh, 25 µl, 50 times diluted) (c). Tomato was chopped into small pieces and homogenized in Assay buffer (1 g/ml). Homogenate was incubated for a minimum of 10 min. at room temperature. Centrifuged (10000 rpm; 4C; 10 min) and supernatant was collected. Supernatant was diluted using Assay Buffer. Recommended dilution (30-120 fold). Sample volume can range between 2 and 50 ul (diluted samples). **Note:** A blender may improve glutamate extraction from tomato (Optional). Enzyme mix is inhibited by tomato homogenate. User must dilute tomato sample. Assays were performed following the kit protocol.

**RELATED PRODUCTS:**

| | |
|---|---|
| NAD/NADH Quantification Kit | NADP/NADPH Quantification Kit |
| ADP/ATP Ratio Assay Kit | Ascorbic Acid Quantification Kit |
| Glucose Assay Kit | Fatty Acid Assay Kit |

***FOR RESEARCH USE ONLY! Not to be used on humans.***

## GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer | • Assay buffer must be at room temperature |
| | • Omission of a step in the protocol | • Refer and follow the data sheet precisely |
| | • Plate read at incorrect wavelength | • Check the wavelength in the data sheet and the filter settings of the instrument |
| | • Use of a different 96-well plate | • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type | • Refer data sheet for details about incompatible samples |
| | • Samples prepared in a different buffer | • Use the assay buffer provided in the kit or refer data sheet for instructions |
| | • Samples were not deproteinized (if indicated in datasheet) | • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated |
| | • Cell/ tissue samples were not completely homogenized | • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope |
| | • Samples used after multiple free-thaw cycles | • Aliquot and freeze samples if needed to use multiple times |
| | • Presence of interfering substance in the sample | • Troubleshoot if needed, deproteinize samples |
| | • Use of old or inappropriately stored samples | • Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components | • Thaw all components completely and mix gently before use |
| | • Use of expired kit or improperly stored reagents | • Always check the expiry date and store the components appropriately |
| | • Allowing the reagents to sit for extended times on ice | • Always thaw and prepare fresh reaction mix before use |
| | • Incorrect incubation times or temperatures | • Refer datasheet & verify correct incubation times and temperatures |
| | • Incorrect volumes used | • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components | • Thaw and resuspend all components before preparing the reaction mix |
| | • Pipetting errors in the standard | • Avoid pipetting small volumes |
| | • Pipetting errors in the reaction mix | • Prepare a master reaction mix whenever possible |
| | • Air bubbles formed in well | • Pipette gently against the wall of the tubes |
| | • Standard stock is at an incorrect concentration | • Always refer the dilutions in the data sheet |
| | • Calculation errors | • Recheck calculations after referring the data sheet |
| | • Substituting reagents from older kits/ lots | • Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength | • Check the equipment and the filter setting |
| | • Samples contain interfering substances | • Troubleshoot if it interferes with the kit |
| | • Use of incompatible sample type | • Refer data sheet to check if sample is compatible with the kit or optimization is needed |
| | • Sample readings above/below the linear range | • Concentrate/ Dilute sample so as to be in the linear range |
| **Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems. | | |