Product Information

Glutamate Colorimetric Assay Kit

I. Kit Contents:

Components	K2133-100 100 assays	Cap Color	Part Number
Glutamate Assay Buffer	25 ml	WM	K2133-C-1
Glutamate Enzyme Mix	1 vial	Green	K2133-C-2
Glutamate Developer	1 vial	Red	K2133-C-3
Glutamate Standard (0.1M)	0.1 ml	Yellow	K2133-C-4

II. Introduction:

Glutamate is an acidic proteinogenic amino acid and is a non-essential amino acid. Glutamate is an important molecule in cellular metabolism. Glutamate is a critical excitatory neurotransmitter in the mammalian nervous system. In humans, glutamate is involved in disposal of excess or waste nitrogen and amino acid degradation. Glutamate is also involved in memory and learning, and is associated with diseases like lathyrism, amyotrophic lateral sclerosis, Alzheimer's disease, some forms of mental retardation and autism. Glutamic acid is also present in many foods and is used as a flavor enhancer in food industry.

The Glutamate Colorimetric Assay Kit provides a sensitive, fast and convenient way for detection of glutamate in various samples based on colorimetric method. The Glutamate Enzyme Mix recognizes glutamate as a specific substrate leading to proportional color development, which can be detected by colorimetric (spectrophotometry at $\lambda = 450$ nm) method.

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 (1×10^6) 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
Assay Buffer	90 µl	92 µl
Glutamate Developer	8 µl	8 µl
Glutamate Enzyme Mix	2 µl	---

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 = Sa/Sv nmol/µl, or mM

Where: Sa is the sample amount of unknown (in nmol) from standard curve,

Sv is sample volume (µl) added into the wells.

L-Glutamic acid Molecular Weight is 147.13 g/mol.

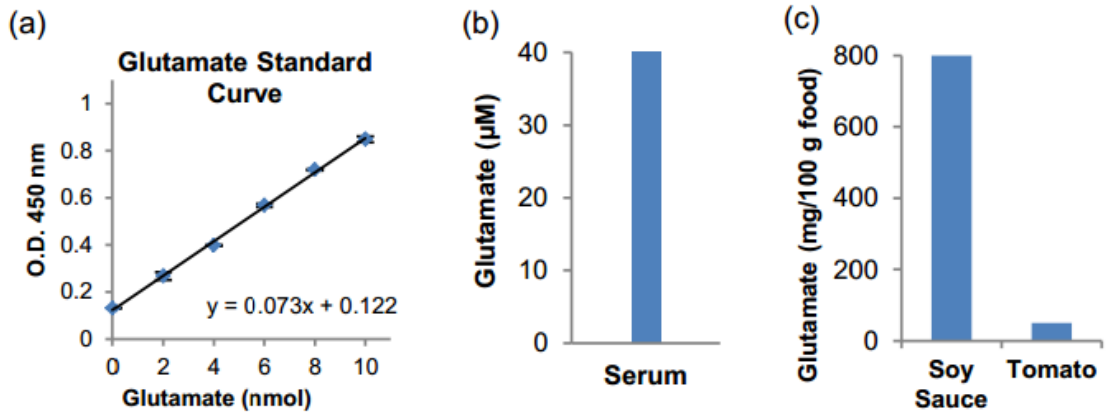

Figure. Glutamate Standard Curve (a). Glutamate concentration in human serum (25 µl) (b), and soy sauce (25 µl, 500 times diluted) and tomato (fresh, 25 µl, 50 times diluted) (c). Tomato was chopped into small pieces and homogenized in Assay buffer (1 g/ml). Homogenate was incubated for a minimum of 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.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

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

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Our promise

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