3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Glutamate Assay Kit

Catalog Number **MAK004**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glutamate, one of the two acidic proteinogenic amino acids, is also a key molecule in cellular metabolism. The synthesis of α-ketoglutarate from glutamate by glutamate dehydrogenase is a key TCA cycle anaplerotic reaction. In humans, glutamate plays an important role both in amino acid degradation and disposal of excess or waste nitrogen. Within the mammalian nervous system, glutamate is the most abundant excitatory neurotransmitter. It is believed to be involved in learning and memory, and may also be involved in diseases like amyotrophic lateral sclerosis, autism, some forms of mental retardation, and Alzheimer's disease. Glutamate is present in a wide variety of foods and has been used as a flavor enhancer in food industry.

Glutamate concentration is determined by an enzymatic assay, which results in a colorimetric (450 nm) product, proportional to the glutamate present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Glutamate Assay Buffer Catalog Number MAK004A	25 mL
Glutamate Enzyme Mix Catalog Number MAK004B	1 vl
Glutamate Developer Catalog Number MAK004C	1 vl
Glutamate Standard, 0.1 M Catalog Number MAK004D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glutamate Assay Buffer – Allow buffer to come to room temperature before use.

Glutamate Enzyme Mix – Reconstitute in 220 µL of Glutamate Assay Buffer. Mix well by pipetting. Aliquot enough of reconstituted Enzyme Mix for use in assay (2 µL per assay well), and then immediately aliquot and store the remainder at –20 °C. Keep the enzyme mix on ice until use in assay. The enzyme mix remains active for at least 2 months after reconstitution when stored at –20 °C in a frost-free freezer.

Glutamate Developer – Reconstitute in 820 µL of water. Mix well by pipetting (do not vortex). Aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glutamate Standards for Colorimetric Detection
Dilute 10 µL of the 0.1 M Glutamate Standard with 990 µL of the Glutamate Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glutamate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue or cells (1×10^6) can be homogenized in 100 µL of the Glutamate Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Serum samples (10–50 µL) can be directly added to wells.

Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if enzymes in the samples interfere with the assay. If protein, fat, or solids/particulates are present, samples should be filtered through a 10 kDa MWCO spin filter.

Bring samples to a final volume of 50 µL with Glutamate Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Include a blank sample for each sample by omitting the Glutamate Enzyme Mix in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 100 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Blank Sample	Samples and Standards
Glutamate Assay Buffer	92 µL	90 µL
Glutamate Developer	8 µL	8 µL
Glutamate Enzyme Mix	-	2 µL

2. Add 100 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) glutamate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate glutamate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample reading from the sample reading to obtain the corrected measurement for each sample. Using the corrected measurement, the amount of glutamate present in the sample may be determined from the standard curve.

Concentration of Glutamate

$$S_a/S_v = C$$

S_a = Amount of glutamate in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of glutamate in sample

Glutamate molecular weight: 147.3 g/mole

Sample Calculation

Amount of glutamate (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of glutamate in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 147.3 \text{ ng/nmole} = 17.2 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Glutamate Assay Buffer
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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