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 Oxidase Assay Kit

Catalog Number **MAK170**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Glutamate oxidase is an oxidoreductase that catalyzes the oxidative deamination of L-glutamate to ketoglutarate, ammonia, and hydrogen peroxide.

The Glutamate Oxidase Assay Kit provides a simple and direct procedure for measuring glutamate oxidase activity in a variety of samples such as solutions and cell extracts. Glutamate Oxidase activity is determined by a coupled enzyme assay, in which the oxidation of glutamic acid results in a fluorescent product (λ_{ex} =540/λ_{em} = 590 nm) proportional to the glutamate oxidase activity present. One unit of glutamate oxidase activity is the amount of enzyme that catalyzes the conversion of L-glutamic acid to 1.0 μmole of α-ketoglutaric acid per minute at pH 7.4 at room temperature.

Components
The kit is sufficient for 200 assays in 96 well plates.

Red Fluorescent Probe Catalog Number MAK170A	1 vl
Assay Buffer Catalog Number MAK170B	20 mL
Horseradish Peroxidase Catalog Number MAK170C	1 vl
Glutamic Acid Catalog Number MAK170D	1 vl
Glutamate Oxidase Catalog Number MAK170E	1 vl
DMSO Catalog Number MAK170F	0.2 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

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.

Storage/Stability
The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

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

Allow all reagents to come to room temperature before use.

Red Fluorescent Probe – Reconstitute with 40 μL of DMSO to make a 250× Red Fluorescent Probe stock solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Horseradish Peroxidase (HRP) – Reconstitute with 200 μL of Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Glutamic Acid – Reconstitute with 1.0 mL of water. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Glutamate Oxidase – Reconstitute with 100 μL of Assay Buffer to prepare a 150 milliunits/mL stock solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Procedure
All samples and standards should be run in duplicate.

Glutamate Oxidase Standards
Add 30 µL of the 150 milliunits/mL Glutamate Oxidase stock solution to 420 µl of Assay Buffer to prepare a 10 milliunits/mL standard solution. Take 150 µL of the 10 milliunits/mL standard solution and prepare serial dilutions with Assay Buffer generating 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0 (blank) milliunits/mL standards. Add 50 µL of each serially diluted standard into appropriate wells of a 96 well plate.

Sample Preparation
Add up to 50 µL of sample to wells. Bring samples to a final volume of 50 µL with Assay Buffer. The red fluorescent probe stock solution is not stable in the presence of thiols (such as 2-mercaptoethanol and DTT) or at high pH (>8.5). The final concentration of thiols in the reaction mix should be <10 µM. The reaction should be performed at pH 7–8.

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

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Red Fluorescent Probe	20 µL
HRP	12.5 µL
Glutamic Acid	12.5 µL
Assay Buffer	5 mL

Note: The Master Reaction Mix is enough for one plate and can be scaled if necessary.

2. Add 50 µL of the Master Reaction Mix to each of the sample, blank, and standard wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30–60 minutes at room temperature. Cover the plate during the incubation.

3. Measure the fluorescence intensity (λ_{ex} =540/ λ_{em} = 590 nm (cut off at 570 nm).

Note: This assay can be adapted for use with 384 well plates. When working with 384 well plates, add 25 µL of standard, sample, working solution, and Master Reaction Mix to each well at the respective steps.

Results
Calculations
The background blank for the assay is the value obtained for the 0 (blank) glutamate oxidase 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 standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

The amount of glutamate oxidase activity present in the samples may be determined from the standard curve. One unit of glutamate oxidase activity is the amount of enzyme that catalyzes the conversion of L-glutamic acid to 1.0 µmole of α-ketoglutaric acid per minute at pH 7.4 at room temperature.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold Reagents	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 fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in an incompatible buffer	Make sure the assay buffer has a pH between 7–8 and that thiols are present at <10 µM.
	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 needed to use 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
	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
	Samples measured at incorrect wavelength	Check the equipment and filter settings
Unanticipated results	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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