Amplite™ Fluorimetric Glutamate Oxidase Assay Kit *Red Fluorescence*

Catalog number: 11302
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: Horseradish Peroxidase (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Glutamic Acid	Freeze (<-15 °C), Minimize light exposure	3.4 mg
Component E: Glutamate Oxidase Standard (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial (15 mU, lyophilized)
Component F: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Glutamate oxidase belongs to the family of oxidoreductases, specifically those acting on the CH-NH2 group of donors with oxygen as an acceptor. It is an enzyme that specifically catalyzes the oxidative deamination of L-glutamate in the presence of water and oxygen with the formation of o-ketoglutarate, ammonia, and hydrogen peroxide. The Amplite™ Fluorimetric Glutamate Oxidase Assay Kit provides a quick and ultrasensitive method for the measurement of glutamate oxidase in solution and in cell lysates. In the assay, L-glutamic acid is oxidized to ?-ketoglutarate, NH3 and H2O2 by glutamate oxidase. L-Alanine and L-glutamate-pyruvate transaminase are included in the reaction, resulting in multiple cycles of the initial reaction, thus significantly amplifying the production of H2O2. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader or an absorbance microplate reader. With the Amplite™ Glutamate Oxidase Assay kit, we have detected as little as 40 ?U/mL glutamate oxidase in a 100 µL reaction volume. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

AT A GLANCE

Protocol summary

1. Glutamate Oxidase standards or test samples (50 µL)
2. Add Glutamate Oxidase working solution (50 µL)
3. Incubate at room temperature for 30 - 60 min
4. Read fluorescence intensity at Ex/Em = 540/590 nm

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red stock solution (250X):
Add 40 µL of DMSO (Component F) into the vial of Amplite™ Red (Component A). The stock solution should be used promptly.

Note The Amplite™ Red is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite™ Red is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH 7 - 8. The provided assay buffer, pH 7.4, is recommended.

2. HRP stock solution (400X):
Add 200 µL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).

3. Glutamic Acid stock solution (400X):
Add 1.0 mL of ddH_2O into the vial of Glutamic Acid (Component D) to make 400X glutamic acid stock solution.

4. Glutamate Oxidase (GO) standard solution (150 mU/mL):
Add 100 µL of Assay Buffer (Component B) into the vial of Glutamate Oxidase Standard (lyophilized, Component E) to make 150 mU/mL Glutamate Oxidase (GO) standard solution.

PREPARATION OF STANDARD SOLUTION

Glutamate Oxidase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11302

Add 30 µL of 150 mU/mL GO standard solution into 420 µL of Assay Buffer (Component B) to get 10 mU/mL GO standard solution (GO7). Take 10 mU/mL GO standard solution to perform 1:3 serial dilutions to get remaining serially diluted GO standards (GO6 - GO1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Amplite™ Red stock solution (250X), 12.5 µL of HRP stock solution (400X) and 12.5 µL of Glutamic Acid stock solution (400X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.07 mL Glutamate Oxidase working solution(GO working solution). Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of GO standards and test samples in a solid black 96-well microplate. GO= glutamate oxidase standards (GO1 - GO7, 0.01 to 10 mU/mL), BL=blank control, TS=test samples.

BL	BL	TS	TS
GO1	GO1	...	...
GO2	GO2	...	...
GO3	GO3		
GO4	GO4		
GO5	GO5		
GO6	GO6		
GO7	GO7		

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Table 2. Reagent composition for each well. Higher concentrations of GO may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red (to a non-fluorescent product).

Well	Volume	Reagent
GO1 - GO7	50 µL	Serial Dilution (0.01 to 10 mU/mL)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

1. Prepare GO standards (GO), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of GO working solution to each well of GO standard, blank control, and test samples to make the total GO assay volume of 100 µL/well. For a 384-well plate, add 25 µL of GO working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm), cutoff = 570 nm.

Note The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Glutamate Oxidase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

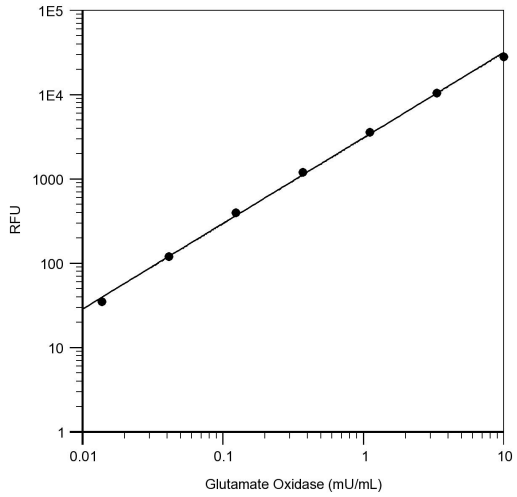

Figure 1. Glutamate oxidase dose response was measured with Amplite™ Fluorimetric Glutamate Oxidase Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER