# Amplite™ Fluorimetric Glutamate Oxidase Assay Kit

**Red Fluorescence**

| *Ordering Information:* | *Storage Conditions:* | *Instrument Platform:* |
|---|---|---|
| Product Number: 11302 (200 assays) | Keep at -20 °C and protect from light | Fluorescence microplate readers |

## Introduction

Glutamate oxidase specifically catalyzes the oxidative deamination of L-glutamate in the presence of water and oxygen with the formation of ketoglutarate, ammonia, and hydrogen peroxide. Glutamate oxidase can be used as an analytic reagent and as a basis for developing biosensors for the determination of L-glutamate, L-glutamine, ammonia, and creatinine. These biosensors can be used in clinical biochemistry for the determination of glutamate-pyruvate transaminase and glutamate-oxalacetate transaminase in biological fluids, which makes the early diagnosis of heart and liver diseases possible.

The Amplite™ Glutamate Oxidase Assay Kit provides a quick and an ultrasensitive method for the measurement of glutamate oxidase in solution and in cell lysates. In the assay, L-glutamic acid is oxidized to α-ketoglutarate, $NH_3$ and $H_2O_2$ by glutamate oxidase. The kit uses our Amplite™ Red substrate which can react with $H_2O_2$ when catalyzed by horseradish peroxidase (HRP) to generate the highly fluorescent product. The signal can be read with either a fluorescence microplate reader at Ex/Em = 530-570 nm/590 -600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576±5 nm. With the Amplite™ Glutamate Oxidase Assay kit, we have detected as little as 40 μU/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.

## Kit Components

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

## Assay Protocol for One 96-Well Plate

**Brief Summary**

**Glutamate Oxidase standards or test samples (50 μL) → Add Glutamate Oxidase assay mixture (50 μL) → Incubate at room temperature for 30-60 min → Read fluorescence intensity at Ex/Em = 540/590 nm**

*Note: Thaw all the kit components to room temperature before starting the experiment.*

**1. Prepare stock solutions:**

1.1 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. Any remaining solution should be aliquoted and frozen at -20 °C.
*Note 1: Avoid repeated freeze-thaw cycles.*
*Note 2: 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*

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*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.*

1.2 HRP stock solution (400X): Add 200 μL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).
*Note: The unused HRP stock solution should be divided into single use aliquots and stored at -20 °C.*

1.3 Glutamic Acid stock solution (400X): Add 1.0 mL of dd$H_2O$ into the vial of Glutamic Acid (Component D) to make 400X glutamic acid stock solution.
*Note: The unused glutamic acid stock solution should be divided into single use aliquots and stored at -20 °C.*

1.4 150 mU/mL Glutamate Oxidase (GO) solution: 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) solution.
*Note: The unused glutamate oxidase solution should be divided into single use aliquots and stored at -20 °C.*

**2. Prepare assay mixture:**

Prepare assay mixture according to the following tables and protect from light.

**Table 1**. Assay mixture for one 96-well plate (2X)

| **Components** | **Volume** |
|---|---|
| Amplite™ Red stock solution (250X, from Step 1.1) | 20 μL |
| HRP (400X, from Step 1.2) | 12.5 μL |
| Glutamic Acid (400X, from Step 1.3) | 12.5 μL |
| Assay Buffer (Component B) | 5 mL |
| Total volume | 5.07 mL |

**3. Prepare serially diluted GO standards (0 to 10 mU/mL):**

3.1 Add 30 μL of 150 mU/mL GO stock solution (from Step 1.4) into 420 μL of Assay Buffer (Component B) to get 10 mU/mL GO standard solution.

3.2 Take 150 μL of 10 mU/mL GO standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.0, 1 and 0 mU/mL serially diluted GO standards.

3.3 Add GO standards and/or GO-containing test samples into a black wall/solid bottom 96-well microplate as described in Tables 2 and 3

**Table 2**. Layout of GO standards and test samples in a solid black 96-well microplate

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BL | BL | TS | TS | …. | …. | | | | | | |
| GO1 | GO1 | …. | …. | …. | …. | | | | | | |
| GO2 | GO2 | | | | | | | | | | |
| GO3 | GO3 | | | | | | | | | | |
| GO4 | GO4 | | | | | | | | | | |
| GO5 | GO5 | | | | | | | | | | |
| GO6 | GO6 | | | | | | | | | | |
| GO7 | GO7 | | | | | | | | | | |

*Note: GO= glutamate oxidase standards, BL=blank control, TS=test samples.*

**Table 3**. Reagent composition for each well

| **GO Standard** | **Blank Control** | **Test Sample** |
|---|---|---|
| Serial Dilutions* (50 μL) | Assay Buffer (Component B): 50 μL | 50 μL |

**Note 1: Add the serially diluted glutamate oxidase standards from 0.01 mU/mL to 10 mU/mL into each well from GO1 to GO7 in duplicate.*

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*Note 2: High concentration of GO may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red (to a non-fluorescent product).*

**4. Run GO assay:**

4.1 Add 50 µL of assay mixture (from Step 2) into each well of the GO standard, blank control, and test samples (see Step 3, Table 2) to make the total GO assay volume of 100 µL/well.
*Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay mixture into each well.*

4.2 Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

4.3 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/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.*

**4. Run Data Analysis:**

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with glutamate oxidase reactions. The typical data are shown in Figure 1 (GO standard curve).
*Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

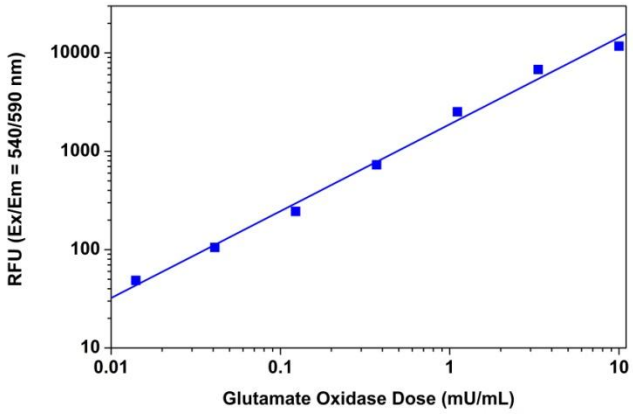


**Figure 1.** Glutamate oxidase dose response was measured with Amplite™ Fluorimetric Glutamate Oxidase Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 40 µU/mL glutamate oxidase was detected with 60 minutes incubation time (n=3).

**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.**