Amplite™ Fluorimetric Glucose Oxidase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11300 (500 assays)	Keep at -20 °C and protect from light	Fluorescence microplate readers

Introduction

The glucose oxidase is a dimeric protein that catalyzes the oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, which is hydrolyzed to gluconic acid. It is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages, food and other agricultural products. Furthermore, Glucose oxidase is commonly used in biosensors to detect glucose.

The Amplite™ Glucose Oxidase Assay Kit provides a quick and sensitive method for the measurement of glucose oxidase in solution. It can be performed in a convenient 96-well or 384-well microtiter plate format and readily adapted to automation without a separation step. 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 at Ex/Em = 540/590 nm or an absorbance microplate reader at 576 nm. With the Amplite™ Fluorimetric Glucose Oxidase Assay Kit, we have detected as little as 0.05 mU/mL glucose oxidase in a 100 µL reaction volume.

Kit Key Features

Sensitive:	Detect as low as 0.05 mU/mL glucose oxidase in solution.
Continuous:	Readily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**
Component A: Amplite™ Red (light-sensitive)	1 vial
Component B: Assay Buffer	1 bottle (50 mL)
Component C: Horseradish Peroxidase (HRP)	1 vial
Component D: Glucose Oxidase	1 vial (100 units)
Component E: DMSO	1 vial (200 µL)
Component F: Glucose	1 vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add glucose oxidase standards or test samples (50 µL) → Incubate at 37 °C for 10-30 minutes → Monitor 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 250X Amplite™ Red stock solution: Add 100 µL of DMSO (Component E) into the vial of Amplite™ Red (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen 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

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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.

1.2 50X HRP stock solution: Add 1 mL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).
Note: The unused 50X HRP stock solution should be divided into single use aliquots and stored at -20°C.

1.3 100 U/mL glucose oxidase stock solution: Add 1 mL of Assay Buffer (Component B) into the vial of Glucose Oxidase (Component D).
Note: The unused 100 U/mL glucose oxidase stock solution should be divided into single use aliquots and stored at -20°C.

1.4 10X glucose stock solution: Add 5 mL of Assay Buffer (Component B) into the vial of Glucose (Component F).
Note: The unused 10X glucose stock solution should be stored at -20°C.

2. Prepare assay reaction mixture:

Prepare assay reaction mixture according to the following tables, protected from light.

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

Components	**Volume**
250X Amplite™ Red Stock Solution (from Step 1.1)	20 μL
50X HRP Stock Solution (from Step 1.2)	100 μL
10X Glucose Stock Solution (from Step 1.4)	500 μL
Assay Buffer (Component B)	4.3 mL
Total volume	5 mL

Table 2 Layout of glucose oxidase standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
GOS1	GOS1	….	….	….	….						
GOS2	GOS2										
GOS3	GOS3										
GOS4	GOS4										
GOS5	GOS5										
GOS6	GOS6										
GOS7	GOS7										

Note: GOS = Glucose Oxidase Standards, BL = Blank Control, TS = Test Samples.

Table 3. Reagent composition for each well

Glucose Oxidase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 μL	Assay Buffer (Component B): 50 μL	50 μL

**Note 1: Add the serially diluted glucose oxidase standards from 0.01 to 10 mU/mL into each well from GOS1 to GOS7 in duplicate.*
Note 2: High concentration of glucose oxidase (e.g., 100 mU/mL, final concentration) may cause reduced fluorescence signal due to the overoxidation of Amplite™ Red (to a non-fluorescent product).

3. Run Glucose oxidase assay:

3.1 Prepare a glucose oxidase standard by diluting 2 μL of the 100 U/mL glucose oxidase stock solution (from Step 1.3) into 200 μL of Assay Buffer (Component B) to have 1000 mU/mL glucose oxidase standard solution. And then take 10 μL of 1000 mU/mL glucose oxidase standard solution to perform 1:100 and 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 mU/mL serially diluted glucose oxidase standards (50 μL/well). A non-glucose oxidase buffer is included as blank control. The final glucose oxidase concentrations should be twofold lower (i.e., 0 to 5 mU/mL).

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

3.3 Incubate the reaction for 10 to 30 minutes at 37 °C, protected from light.
3.4 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 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.

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 glucose oxidase reactions. A glucose oxidase standard curve is shown in Figure 1.
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.

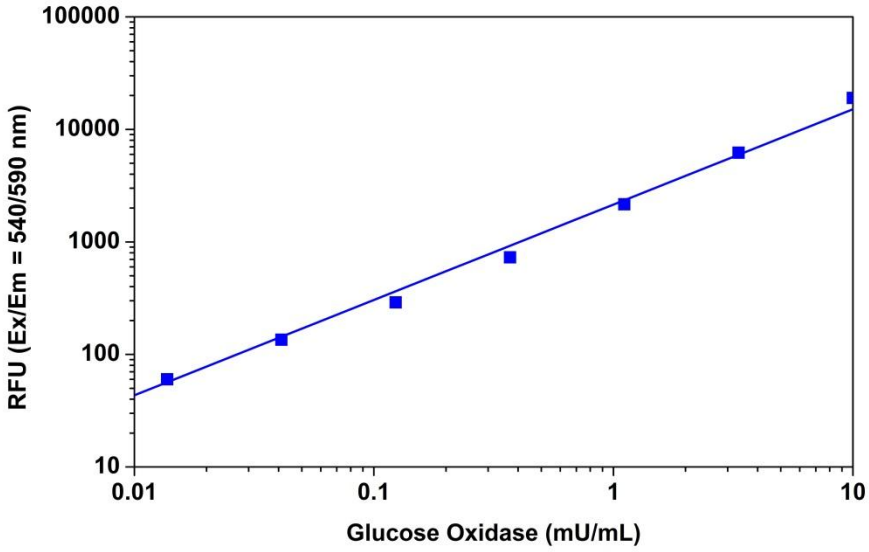

Figure 1. Glucose oxidase dose response was measured with Amplite™ Fluorimetric Glucose Oxidase Assay Kit in a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.05 mU/ mL glucose oxidase was detected with 30 minutes incubation (n=3).

References

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