Amplite™ Fluorimetric Glucose Oxidase Assay Kit *Red Fluorescence*

Catalog number: 11300
Unit size: 500 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)	1 bottle (50 mL)
Component C: Horseradish Peroxidase (HRP)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Glucose Oxidase	Freeze (< -15 °C), Minimize light exposure	1 vial (100 units)
Component E: DMSO	Freeze (< -15 °C)	1 vial (200 µL)
Component F: Glucose	Freeze (< -15 °C), Minimize light exposure	1 vial

OVERVIEW

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 is easily 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 or an absorbance microplate reader. 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.

AT A GLANCE

Protocol Summary

1. Prepare glucose oxidase standards or test samples (50 µL)
2. Add GO working solution (50 µL)
3. Incubate at 37 °C for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

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 100 µL of DMSO (Component E) 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 (50X)
Add 1 mL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).

3. Glucose oxidase standard solution (100 U/mL)
Add 1 mL of Assay Buffer into the vial of Glucose Oxidase (Component D).

4. Glucose stock solution (10X)
Add 5 mL of Assay Buffer into the vial of Glucose (Component F).

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11300

Glucose Oxidase standard
Prepare a glucose oxidase standard by diluting 2 µL of the 100 U/mL glucose oxidase standard solution 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 and perform 1:100 dilution to obtain 10 mU/mL glucose oxidase standard solution (GOS7). Then perform 1:3 serial dilutions to get remaining serially diluted glucose oxidase standards (GOS6-GOS1). 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). Note: High concentrations of glucose oxidase may cause reduced fluorescence signal due to the overoxidation of Amplite™ Red (to a non-fluorescent product).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Amplite™ Red stock solution (250X), 100 µL of HRP stock solution (50X), and 500 µL of Glucose stock solution (10X) into 4.4 mL of Assay Buffer (Component B) to make a total volume of 5 mL Glucose Oxidase (GO) working solution. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of glucose oxidase standards and test samples in a solid black 96-well microplate. GOS = Glucose Oxidase Standard (GOS1-GOS7, 0.01 to 10 mU/mL), BL = Blank Control, TS = Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
GOS1-GOS7	50 µL	serial diltuion (0.01 to 10 mU/mL)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	Sample

1. Prepare glucose oxidase standards (GOS), blank control (BL), and test samples (TS) according ot the layout proided in Table 1 and Table 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 into each well of glucose oxidase standards, blank control, and test samples to make the total glucose oxidase 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 10 to 30 minutes at 37°C, 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). *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. However, the absorption detection will have a lower sensitivity compared to the 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 Glucose 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

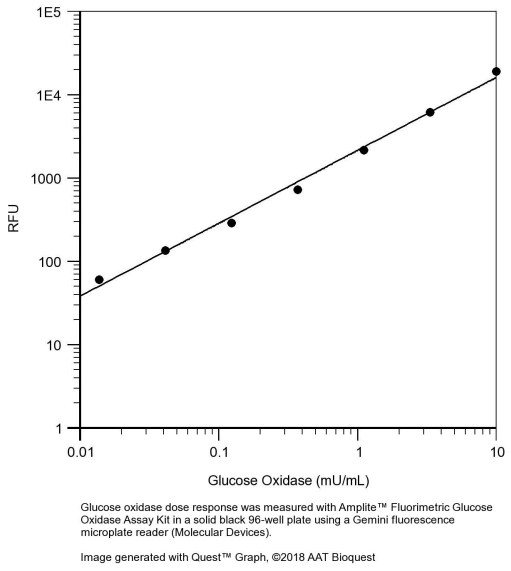

Figure 1. Glucose oxidase dose response was measured with Amplite™ Fluorimetric Glucose Oxidase Assay Kit in a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.