Amplite™ Colorimetric Glucose Oxidase Assay Kit

Catalog number: 11299
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 readily adapted to automation without a separation step. The kit uses our Amplite™ Red substrate which can be monitored using an absorbance microplate reader at 570 nm.

AT A GLANCE

Protocol Summary

1. Prepare glucose oxidase standards or test samples (50 µL)
2. Add working solution (50 µL)
3. Incubate at 37 °C for 10 - 30 minutes
4. Monitor absorbance at OD = 570 nm

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570 nm
Recommended plate	Clear bottom

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).

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

4. Glucose stock solution (10X)
Add 5 mL of Assay Buffer (Component B) 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/11299

Glucose Oxidase standard
Prepare a glucose oxidase standard by diluting 2 µL of the 100 U/mL Glucose Oxidase stock solution into 200 µL of Assay Buffer (Component B) to have 1000 mU/mL glucose oxidase standard solution. Then perform 1:100 serial dilution followed by 1:2 serial dilutions to get serially diluted glucose oxidase standards from 10 mU/mL to 0.156 mU/mL (GOS1 - GOS7).

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 5 mL of working solution.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of glucose oxidase standards and test samples in a clear bottom 96-well microplate. GOS = Glucose Oxidase Standards (GOS1 - GOS7, 0.156 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 Dilution (0.156 to 10 mU/mL)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

1. Prepare glucose oxidase standards (GOS), 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 working solution to each well of glucose oxidase standard, blank control, and test samples to make the total 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 absorbance increase with an absorbance plate reader at OD = 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) 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

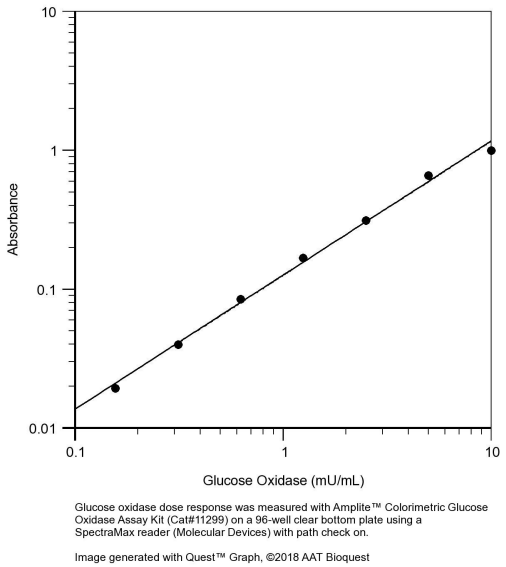

Figure 1. Glucose oxidase dose response was measured with Amplite™ Colorimetric Glucose Oxidase Assay Kit (Cat#11299) on a 96-well clear bottom plate using a SpectraMax reader (Molecular Devices) with path check on.

DISCLAIMER

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