Amplite™ Fluorimetric Glycerol Assay Kit

Catalog number: 13833
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ HRP Substrate (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component D: Glycerol Standard	Freeze (<-15 °C), Minimize light exposure	80 µL/vial
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Glycerol is a precursor for the synthesis of triglycerides and phospholipids in liver and adipose tissue. When fasting, triglycerides stored in these lipid droplets can be hydrolyzed to generate free glycerol and fatty acids. The amount of free glycerol released to the bloodstream is proportional to the triglyceride/fatty acid cycling rate, which is important in the metabolic regulation and heat production. Amplite™ Fluorimetric Glycerol Assay Kit offers a sensitive fluorescence-based assay for measuring glycerol levels in biological samples. This assay is based on an enzyme coupled reaction of glycerol, in which the product hydrogen peroxide can be detected using our Amplite™ HRP substrate in HRP-coupled reactions. The fluorescence signal can be measured by a fluorescence microplate reader. With this Fluorimetric Glycerol Assay Kit, we were able to detect as low as 0.015 mg/L (~0.16 µM) glycerol in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare Glycerol working solution (50 µL)
2. Add Glycerol standards or test samples (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important To achieve the best results, it's strongly recommended to use the black plates. Thaw one vial of each kit component at 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™ HRP Substrate stock solution (200X):
Add 50 µL of DMSO (Component E) into the vial of Amplite™ HRP Substrate (Component A) to make 200X Amplite™ HRP Substrate stock solution. Protect from light.

2. Glycerol standard solution (1 mg/mL):
Add 1 mL of ddH2O or 1x PBS buffer into the vial of Glycerol Standard (Component D) to make 1 mg/mL Glycerol standard solution.

PREPARATION OF STANDARD SOLUTION

Glycerol standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13833

Add 10 µL of 1 mg/mL Glycerol standard solution into 990 µL 1x PBS buffer to generate 10 µg/mL Glycerol standard solution (GS7). Take 10 µg/mL Glycerol standard solution (GS7) and perform 1:3 serial dilutions in 1x PBS buffer to get serially diluted Glycerol standards (GS6-GS1).

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component C) into a bottle of Enzyme Mix (Component B), and mix welll.

2. Add 25 µL of 200X Amplite™ HRP Substrate stock solution into the bottle of Component B+C, and mix well to make Glycerol working solution.

Note This Glycerol working solution is enough for one 96-well plate. It is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Glycerol standards and test samples in a solid black 96-well microplate. GS= Glycerol Standards (GS1 - GS7, 0.01 to 10 µg/mL), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
GS1	GS1	...	...
GS2	GS2	...	...
GS3	GS3		
GS4	GS4		
GS5	GS5		
GS6	GS6		
GS7	GS7		

Table 2. Reagent composition for each well

Well	Volume	Reagent
GS1 - GS7	50 µL	Serial Dilutions (0.01 to 10 µg/mL)
BL	50 µL	1X PBS Buffer
TS	50 µL	test sample

1. Prepare Glycerol standards (GS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25

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µL of reagent per well instead of 50 µL.

2. Add 50 µL of Glycerol working solution to each well of Glycerol standard, blank control, and test samples to make the total Glycerol assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Glycerol working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

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 Glycerol 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

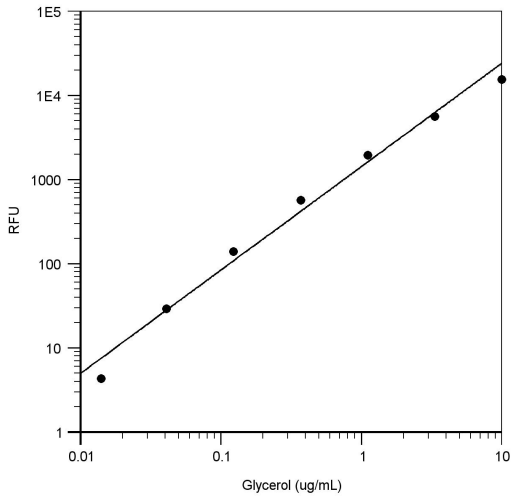

Figure 1. Glycerol was measured with Amplite™ Fluorimetric Glycerol Assay Kit on a solid black 96-well plate using a Gemini microplate reader.

DISCLAIMER