Amplite™ Colorimetric Glycerol Assay Kit

Catalog number: 13832
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red HRP substrate (light sensitive)	Freeze (<-15 °C), Dessicated, 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 synthesis of triglycerides and phospholipids in the 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™ Colorimetric Glycerol Assay Kit offers a sensitive 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™ Red HRP substrate in the HRP-coupled reactions. The signal can be measured with an absorbance microplate reader using OD 575 nm. With this Colorimetric Glycerol Assay Kit, we were able to detect as low as 0.15 µg/mL (~1.6 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 30 min to 1 hour
4. Monitor OD at 575 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:	Absorbance microplate reader
Absorbance:	575 nm
Instrument specification(s):	Path check
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™ HRP substrate stock solution (200X):
Add 50 µL of DMSO (Component E) into the vial of Amplite™ HRP substrate (Component A) to make 200X stock solution.

2. Glycerol standard solution:
Add 1 mL of ddH_2O 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/13832

Add 10 µL of glycerol standard stock solution (1 mg/mL) into 990 µL 1X PBS buffer to generate 10 µg/mL standard solution (G7). Then perform 1:2 serial dilutions to get serially diluted glycerol standards (G6 - G1).

PREPARATION OF WORKING SOLUTION

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

2. Add 25 µL of Amplite™ HRP substrate stock solution into the bottle of Component B + C and mix them well to make glycerol working solution (Component A + B + C).

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

Note One can divide unused Component B+C into single use aliquots and stored at -20 °C.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of glycerol standards and test samples in a clear bottom 96-well microplate. G= Glycerol Standards (G1 - G7, 0.156 to 10 µg/mL), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
G1	G1	...	...
G2	G2	...	...
G3	G3		
G4	G4		
G5	G5		
G6	G6		
G7	G7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
G1 - G7	50 µL	Serial Dilutions (0.156 to 10 µg/mL)
BL	50 µL	1X PBS Buffer
TS	50 µL	Test Sample

1. Prepare glycerol standards (G), 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.

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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 30 minutes to 1 hour, protected from light.

4. Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 575 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 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

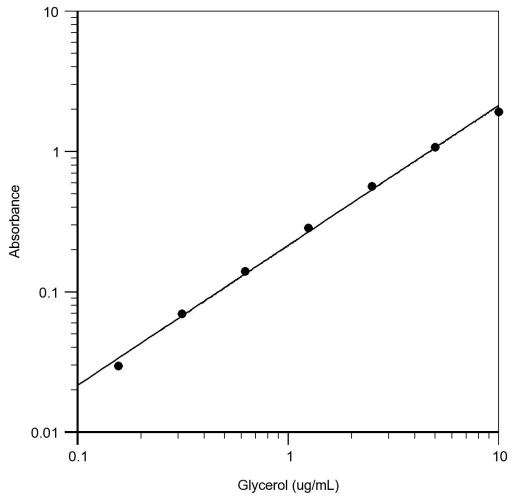

Figure 1. Glycerol dose response was measured with Amplite™ Colorimetric Glycerol Assay Kit on a black wall/clear bottom 96-well plate using a SpectraMax reader.

DISCLAIMER