Amplite™ Colorimetric Glycerol Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13832 (200 tests)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

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. AAT Bioquest's 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 ratio of 570 nm/610 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.

Kit Components

Components	Amount
Component A: Amplite™ Red HRP substrate (light sensitive)	1 vial
Component B: Enzyme Mix	2 bottles (lyophilized powder)
Component C: Assay Buffer	1 bottle (10 mL)
Component D: Glycerol Standard	80 uL/vial
Component E: DMSO	1 vail (100uL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare glycerol assay mixture (50 µL) → Add glycerol standards or test samples (50 µL) → Incubate at room temperature for 30 min to 1 hour→ Monitor OD ratio of 570 nm/610 nm

Note: 1. To achieve the best results, it's strongly recommended to use the black plates.
2. Thaw one vial of each kit component at room temperature before starting the experiment.

1. Prepare Amplite™ HRP substrate stock solution (200X):

Add 50 µL of DMSO into the vial of Amplite™ HRP substrate (**Component A**) to make 200X stock solution.
Note: Make a single used aliquots, and store unused 200 X Amplite™ HRP substrate stock solution at -20ºC, avoid light and repeat freeze-thaw cycles.

2. Prepare glycerol assay mixture:

2.1 Add 5 mL of Assay Buffer (**Component C**) into a bottle of Enzyme Mix (**Component B**), mix them well.
2.2 Add 25 µL of Amplite™ HRP substrate stock solution (from Step 1) into the bottle of **Component B+C** (from Step 2.1), and mix them well to make glycerol assay mixture (**Component A+B+C**).
Note 1. This glycerol assay mixture is enough for one 96-well plate. It is not stable, use it promptly.
*Note 2. One can divide unused **Component B+C** into single use aliquots and stored at -20 °C.*

3. Prepare Glycerol standard stock solution:

Add 1 mL of ddH_2O or 1×PBS buffer into the vial of glycerol standard (**Component D**) to make 1 mg/mL glycerol standard stock solution.
Note: The unused glycerol *standard stock solution should be divided into single use aliquots and stored at -20 °C.*

4. Prepare serial dilutions of glycerol standard (0 to 10 µg/mL):

4.1 Add 10 µL of glycerol standard stock solution (1 mg/mL, from Step 3) into 990 µL 1×PBS buffer to generate 10 µg/mL standard solution.
4.2 And then perform 1:2 serial dilutions to get approximately 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 µg/mL serially diluted glycerol standards.

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4.3 Add serial dilutions of glycerol standard and glycerol containing test samples into a clear bottom 96-well microplate as described in Tables 1 and 2.

Table 1 Layout of glycerol standards and test samples in a clear bottom 96-well microplate

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

Note: G= Glycerol Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

Glycerol Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	1×PBS Buffer : 50 µL	50 µL

**Note: Add the serially diluted glycerol standards from approximately 0.156 µg/mL to 10 µg/mL into wells from G1 to G7 in duplicate.*

5. Run glycerol assay:

5.1 Add 50 µL of glycerol assay mixture (from Step 2.2) to each well of glycerol standard, blank control, and test samples (see Step 4.3) to make the total volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of glycerol assay mixture into each well.

5.2 Incubate the reaction at room temperature for 30 minutes to 1 hour, protected from light.

5.3 Monitor the absorbance increase with an absorbance plate reader with path check at OD of 575 nm.

Data Analysis

The absorbance reading in blank wells (with PBS and glycerol assay mixture only) is used as a control, and is subtracted from the values of those wells with the glycerol standards and test samples. A glycerol standard curve is shown in Figure 1. Calculate the glycerol concentrations of the samples according to the glycerol standard curve.

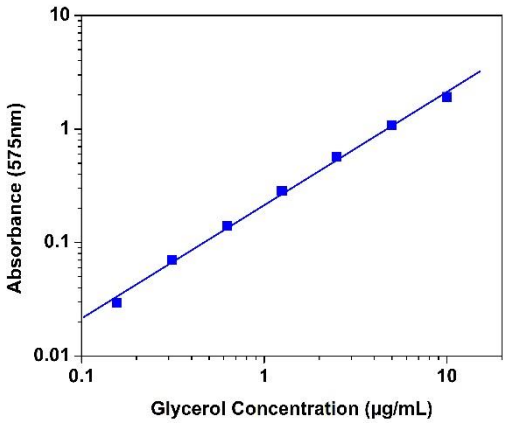

Figure1 Glycerol dose response was measured with Amplite Colorimetric Glycerol Assay Kit on a black wall/clear bottom 96-well plate using a SpectraMax reader. As low as 0.15 µg/mL (~1.6 µM) was detected with 30 minutes incubation.

References

1. Frank MS, Nahata MC, Hilty MD. (1996) Glycerol: A Review of Its Pharmacology, Pharmacokinetics, Adverse Reactions, and Clinical Use. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy.
2. Wolfe RR, Klein S, Carraro F, Weber JM. (1990) Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. American Journal of Physiology-Endocrinology and Metabolism.

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