

**Amplite® Colorimetric Glycerol Assay Kit**

 Catalog number: 13832  
 Unit size: 200 Tests

Component	Storage	Amount (Cat No. 13832)
Component A: Amplite™ Red HRP substrate (light sensitive)	Freeze (< -15 °C), Desiccated, 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 standards or test samples (50 µL)
2. Add glycerol working solution (50 µL)
3. Incubate at room temperature for 30 min to 1 hour
4. Monitor OD at 575 nm

**Important Note**

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**
**Absorbance microplate reader**

Absorbance	575 nm
Recommended plate	Clear bottom
Instrument specification(s)	Path check

**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*

**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. **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.

**Glycerol standard solution**

Add 1 mL of ddH<sub>2</sub>O or 1X PBS buffer into the vial of glycerol standard (Component D) to make 1 mg/mL glycerol standard solution.

**PREPARATION OF STANDARD SOLUTIONS**

For convenience, use the Serial Dilution Planner:

<https://www.aatbio.com/tools/serial-dilution/13832>

**Glycerol standard**

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**

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

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 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 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		

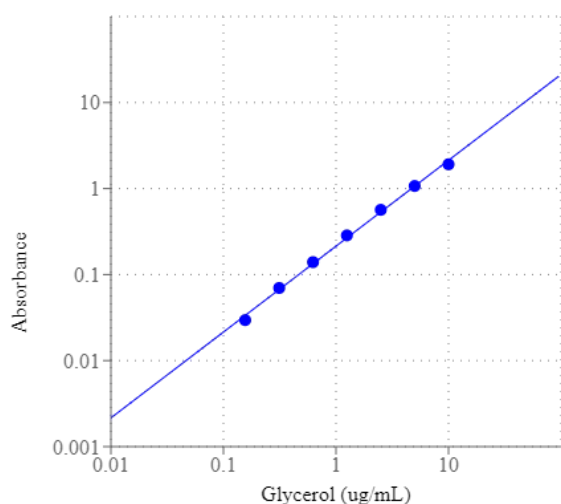
**Table 2.** Reagent composition for each well.

Well	Volume	Reagent
G1 - G7	50 $\mu$ L	Serial Dilutions (0.156 to 10 $\mu$ g/mL)
BL	50 $\mu$ L	1X PBS Buffer
TS	50 $\mu$ L	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  $\mu$ L of reagent per well instead of 50  $\mu$ L.
2. Add 50  $\mu$ L of glycerol working solution to each well of glycerol standard, blank control, and test samples to make the total glycerol assay volume of 100  $\mu$ L/well. For a 384-well plate, add 25  $\mu$ L of glycerol working solution into each well instead, for a total volume of 50  $\mu$ L/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

Placeholder for image details



**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.

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