

## Fluorimetric Glycerol Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13833 (200 tests)	Keep in freezer and protect from light	Fluorescence microplate readers

### Introduction

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. AAT Bioquest's 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 at Ex/Em= 540/590 nm. 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.

### Kit Components

Components	Amount
Component A: Amplite™ 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 vial (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 10-30 min → Monitor fluorescence intensity at Ex/Em = 540/590 nm**

Notes: 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 use aliquots, and store unused 200 X Amplite™ HRP substrate stock solution at -20°C, 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 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 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<sub>2</sub>O 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.

