

Amplite™ Colorimetric Glycerol 3-Phosphate (G3P) Assay Kit

Ordering Information:	Storage Conditions:	Instrument Platform:
Product Number: 13838 (200 assays)	-20°C, avoid light	Absorbance microplate readers

Introduction

Glycerol 3-Phosphate (G3P) is an important intermediate in glycolysis metabolic pathway. Animals, fungi, and plants use G3P to produce ATP. It is used to regenerate NAD⁺ in brain and skeletal muscle cells. G3P has been linked to lipid imbalance diseases such as obesity. Amplite™ G3P Assay Kit provides one of the most sensitive methods for quantifying G3P. The kit uses Amplite™ Red substrate to quantify the concentration of G3P, which is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. The kit is an optimized “mix and read” format that is compatible with HTS applications. It detects as little as 12.5 μM G3P in 100 μL assay volume as shown in Figure 1. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read at ~576±5 nm with an absorbance microplate reader.

Kit Components

Components	Amount
Component A: Amplite™ Red substrate (light sensitive)	1 vial
Component B: Enzyme Mix	2 bottles (lyophilized powder)
Component C: Assay Buffer	1 bottle (10 mL)
Component D: Glycerol 3-Phosphate (G3P) Standard	1 vial (lyophilized powder)
Component E: DMSO	1 vial (100μL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare Glycerol 3-Phosphate assay mixture (50 μL) → Add Glycerol 3-Phosphate standards or test samples (50 μL) → Incubate at RT for 30 min to 1 hour → Monitor absorbance increase at OD of 576 ± 5 nm

Note: Thaw all the kit components at room temperature before starting the experiment.

1. Prepare stock solutions:

- 1.1 Amplite™ Red substrate stock solution (200X): Add 50 μL of DMSO (**Component E**) into the vial of Amplite™ Red substrate (**Component A**) to make a 200X stock solution.
Note: The unused Amplite™ Red substrate stock solution should be divided into single use aliquots. Store at -20 °C and avoid exposure to light.
- 1.2 G3P stock solution: Add 250 μL of ddH₂O into the vial of G3P Standard (**Component D**) to make 10 mM G3P stock solution.
Note: The unused G3P stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare Glycerol 3-Phosphate (G3P) assay mixture:

- 2.1 Add 5 mL of Assay Buffer (**Component C**) to the bottle of Enzyme Mix (**Component B**) and mix well.
- 2.2 Add 25 μL of Amplite™ Red substrate stock solution (200X, from Step 1.1) into the bottle of **Components B** and **C** (from Step 2.1) to make the G3P assay mixture (**Components A, B** and **C**).
Note 1: The G3P assay mixture should be used promptly and kept from light.
*Note 2: One can divide the unused mixture of **Components B** and **C** into single use aliquots and stored at -20 °C.*

3. Prepare serial dilutions of Glycerol 3-Phosphate standard (0 to 200 μM):

- 3.1 Add 100 μL of 10 mM G3P standard stock solution (from Step 1.2) to 900 μL 1×PBS buffer to generate 1000 μM G3P standard solution.
Note: Diluted G3P standard solution is unstable, and should be used within 4 hours.
- 3.2 Take 200 μL of 1000 μM G3P standard (from Step 3.1) to 800 μL 1×PBS buffer to generate 200 μM G3P standard solution, and then perform 1:2 serial dilutions to get 200, 100, 50, 25, 12.5, 6.25, and 0 μM G3P standard.

