

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

 Catalog number: 13838
 Unit size: 200 Tests

Component	Storage	Amount (Cat No. 13838)
Component A: Amplite™ Red Substrate (light sensitive)	Freeze (< -15 °C), 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 3-Phosphate (G3P) Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component E: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

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.

AT A GLANCE
Protocol Summary

1. Prepare Glycerol 3-Phosphate standards or test samples (50 µL)
2. Add Glycerol 3-Phosphate working solution (50 µL)
3. Incubate at RT for 30 min to 1 hour
4. Monitor absorbance increase at OD of 575 nm

Important

Thaw all the kit components at room temperature before starting the experiment

KEY PARAMETERS
Absorbance microplate reader

Absorbance	575 nm
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

Amplite™ Red 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. Avoid exposure to light.

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.

G3P standard solution (10 mM)

Add 250 µL of ddH₂O (Component B) into the vial of G3P Standard (Component D) to make 10 mM G3P standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
<https://www.aatbio.com/tools/serial-dilution/13838>

G3P standard

Add 100 µL of 10 mM G3P standard stock solution to 900 µL 1X PBS buffer to generate 1000 µM G3P standard solution. Take 200 µL of 1000 µM G3P standard to 800 µL 1X PBS buffer to generate 200 µM G3P standard solution (CS7), and then perform 1:2 serial dilutions to get the remaining G3P standards (CS6 - CS1). [note] Diluted G3P standard solution is unstable, and should be used within 4 hours. [/note]

PREPARATION OF WORKING SOLUTION

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

Add 25 µL of Amplite™ Red substrate stock solution (200X) into the same bottle of Enzyme Mix (Components B) to make the G3P working solution (final bottle should contain Components A, B and C).

Note: This working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of G3P standards and test samples in a clear bottom 96-well microplate. CS = G3P standard (CS1 - CS7, 3.12 to 200 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
CS1	CS1
CS2	CS2
CS3	CS3		
CS4	CS4		
CS5	CS5		
CS6	CS6		
CS7	CS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
CS1 - CS7	50 µL	Serial Dilution (3.12 to 200 µM)
BL	50 µL	Assay Buffer (Component C)

TS	50 μ L	Test Sample
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1. Prepare G3P standards (CS), blank controls (BL), and test samples (TS) into a 96-well clear bottom/black wall microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 μ L of reagent per well instead of 50 μ L.

Note: Treat the cells or tissues as desired.

2. Add 50 μ L of G3P working solution to each well of G3P standard, blank control, and test samples to make total G3P assay volume of 100 μ L/well. For a 384-well plate, add 25 μ L of G3P working solution into each well instead, for a total volume of 50 μ L/well.
3. Incubate the reaction at room temperature for 30 min to 1 hour, protected from light.
4. Monitor the absorbance increase with an absorbance microplate reader at 575 nm (or ratio of 570 nm/610 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

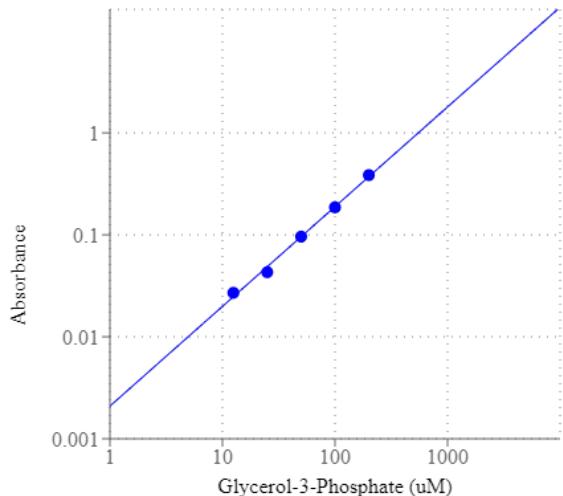


Figure 1. Glycerol 3-phosphate dose response was obtained with Amplate® Colorimetric Glycerol 3-Phosphate (G3P) Assay Kit in a 96-well clear bottom /black wall plate using a SpectraMax absorbance microplate reader (Molecular Devices).

DISCLAIMER

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