

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

Catalog number: 13838

Unit size: 200 Tests

Component	Storage	Amount
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 working solution (50 µL)
2. Add Glycerol 3-Phosphate standards or test samples (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

Instrument:	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.

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. Avoid exposure to light.

2. G3P standard solution (10 mM):

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

PREPARATION OF STANDARD SOLUTION

G3P standard

For convenience, use the Serial Dilution Planner:

<https://www.aatbio.com/tools/serial-dilution/13838>

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.

PREPARATION OF WORKING SOLUTION

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

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

Note The G3P working solution should be used promptly and kept from 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, 6.25 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 (6.25 to 200 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	Test Sample

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 µL of reagent per well instead of 50 µL.

Note Treat the cells or tissues as desired.

2. Add 50 μL of G3P working solution to each well of G3P standard, blank control, and test samples to make total G3P assay volume of 100 μL /well. For a 384-well plate, add 25 μL of G3P working solution into each well instead, for a total volume of 50 μL /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

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Glycerol-3-Phosphate samples. We recommend using the Online Linear Regression Calculator which can be found at:

<https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator>

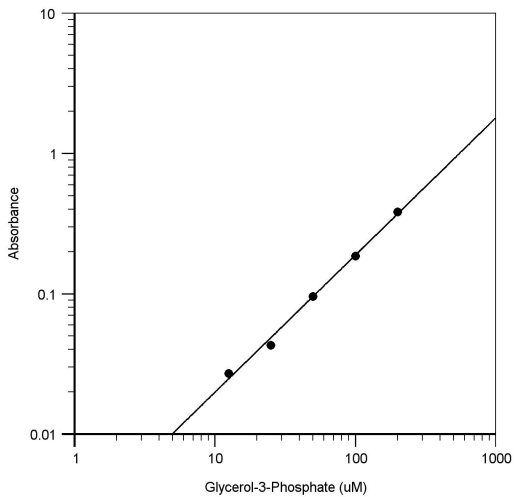


Figure 1. Glycerol 3-phosphate dose response was obtained with Amplitude™ Colorimetric Glycerol 3-Phosphate (G3P) Assay Kit in a 96-well clear bottom /black wall plate using a SpectraMax absorbance microplate reader (Molecular Devices).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.