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

Catalog number: 13837
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 the 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™ Glycerol 3-Phosphate Assay Kit provides one of the most sensitive methods for quantifying G3P. The kit uses Amplite™ Red substrate to quantify G3P concentration, which is proportional to the production of hydrogen peroxide in the G3P oxidase-mediated enzyme coupling reactions. The kit is an optimized "mix and read" format that is compatible with HTS applications. It detects as little as 41 picomole G3P in 100 µL solution (0.41 µM) as shown in Figure 1. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format without a separation step. Its signal can be easily read with a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare G3P working solution (50 µL)
2. Add G3P standards or test samples (50 µL)
3. Incubate at RT for 10 - 30 min
4. Read fluorescence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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. Glycerol 3-Phosphate (G3P) standard solution (10 mM):
Add 250 µL of ddH_2O 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/13837

Add 10 µL of 10 mM G3P standard stock solution to 990 µL 1X PBS buffer to generate 100 µM standard solution (CS7). Then perform 1:3 serial dilutions to get serially diluted 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 bottle of Components B and C to make the G3P working solution (Components A, B and C).

Note Use promptly and keep from light.

Note One can divide unused mixture of Components B and C into single use aliquots and stored at -20 °C.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of G3P standards and test samples in a solid black 96-well microplate. CS = G3P standard (CS1 - CS7, 0.1 to 100 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 cell.

Well	Volume	Reagent
CS1 - CS7	50 µL	serial dilution (0.1 to 100 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	sample

1. Prepare G3P standards (CS), blank controls (BL), and test samples (TS) into a 96-well black microplate according to the layout provided in Table 1 and Table

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

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

3. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

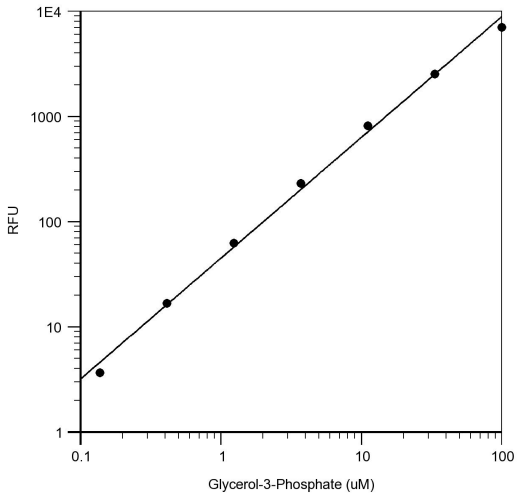

Figure 1. Glycerol-3-phosphate dose response was obtained with Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER