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

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 13837 (200 assays)	-20 °C, avoid light	Fluorescence microplate readers

Introduction

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 Ex/Em = ~540/590 nm.

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 G3P assay mixture (50 µL) → Add G3P standards or test samples (50 µL) → Incubate at RT for 10-30 min → Read fluorescence intensity at Ex/Em = 540/590 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 Glycerol 3-Phosphate (G3P) stock solution: Add 250 µL of ddH_2O 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™ HRP 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 unused mixture of **Components B** and **C** into single use aliquots and stored at -20 °C.*

3. Prepare serial dilutions of Glycerol 3-Phosphate (G3P) standard (0 to 200 µM):

3.1 Add 10 µL of 10 mM G3P standard stock solution (from Step 1.2) to 990 µL 1×PBS buffer to generate 100 µM standard solution.
Note: Diluted G3P standard solution is unstable, and should be used within 4 hours.

3.2 And then perform 1:3 serial dilutions to get approximately 30, 10, 3, 1, 0.3, 0.1 and 0 µM serially diluted G3P standards.

3.3 Add G3P standards and test samples into a 96-well black solid microplate as described in Tables 1 and 2.

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Note: Treat the cells or tissue samples as desired.

Table 1. Layout of G3P standards and test samples in a solid black 96-well microplate*

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

**Note: CS= G3P Standards; BL=Blank Control; TS=Test Samples*

Table 2. Reagent composition for each well*

G3P Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay buffer: 50 µL	50 µL

**Note: Add the serially dilutions of G3P standards from 0.1 to 100 µM into wells from CS1 to CS in duplicate.*

4. Run Glycerol 3-Phosphate (G3P) assay:

4.1 Add 50 µL of G3P assay mixture (from Step 2.2) to each well of the G3P standard, blank control, and test samples (see Step 3.3) to make the total G3P assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL sample and 25 µL of G3P assay mixture per well.

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

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

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the G3P reactions. G3P standard curve is shown in Figure 1. The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

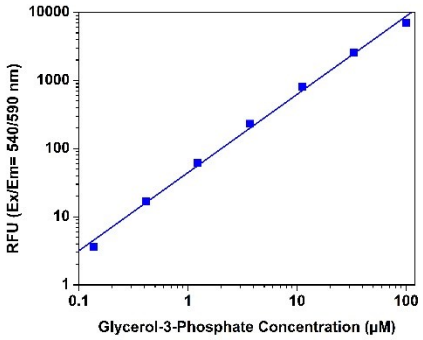

Figure 1. G3P dose response was obtained with Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat#13837) in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.41 µM of Glycerol 3-Phosphate can be detected with 30 minutes incubation time (n=3).

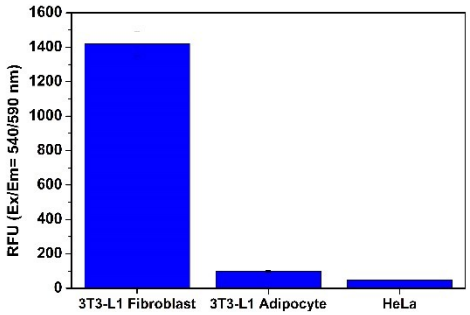

Figure 2. G3P Measurement in 3T3-L1 Adipocyte, 3T3-L1 Fibroblast and HeLa cell lysate with Kit 13837. Cells (1×10^5) were lysed using ReadiUse™ mammalian cell lysis buffer (Cat#20012). 50 µL of Cell lysate were used for testing. Assay were performed following the kit protocol.

References

1. Wendel AA, Lewin TM, Coleman RA. (2008) Glycerol-3-phosphate acyltransferases: Rate limiting enzymes of triacylglycerol biosynthesis. Molecular and Cell Biology of Lipids.