Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit

Catalog number: 13804
Unit size: 200 Tests

Component	Storage	Amount
Component A: Enzyme Probe	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: NADP	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Glucose-6-Phosphate Standard	Freeze (<-15 °C), Minimize light exposure	3.04 mg/vial

OVERVIEW

Glucose-6-phosphate (G6P) is a key intermediate for glucose transport into cells. G6P may also be converted to glycogen or starch for storage in the liver and muscles. G6P is utilized by glucose-6-phosphate dehydrogenase (G6PD) to generate the reducing equivalents in the form of NADPH. This is particularly important in red blood cells where G6PD deficiency leads to hemolytic anemia. AAT Bioquest's Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit provides a simple, sensitive and rapid fluorescence-based method for detecting G6P in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the coupled enzyme assay, the G6P concentration is proportionally related to NADPH that is specifically monitored by a fluorogenic NADPH sensor. The fluorescence signal can be read by a fluorescence microplate reader. With the Amplite™ G6P Assay Kit, we were able to detect as little as 0.3 µM G6P in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare G6P working solution (50 µL)
2. Add G6P standards or test samples (50 µL)
3. Incubate at room temperature for 30 minutes - 2 hours
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw 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
Instrument:	Absorbance microplate reader
Absorbance:	575/605 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. NADP stock solution (100X):
Add 100 µL of H_2O into the vial of NADP (Component C) to make 100X NADP stock solution.

2. G6P standard solution (100 mM):
Add 100 µL of H_2O or 1x PBS buffer into the vial of G6P Standard (Component D) to make 100 mM G6P standard solution.

PREPARATION OF STANDARD SOLUTION

G6P standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13804

Add 10 µL of 100 mM G6P standard solution into 990 µL 1x PBS buffer to generate 1 mM G6P standard solution. Then, add 100 µL of 1 mM G6P standard solution into 900 µL 1 x PBS buffer to make 100 µM G6P standard solution (G6P7). Take 100 µM G6P standard solution (G6P7) and perform 1:3 serial dilutions to get serially diluted G6P standards (G6P6 - G6P1) with 1x PBS buffer.

Note Diluted G6P standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Probe (Component A), and mix well.

2. Add 50 µL of 100X NADP stock solution into the bottle of Component A+B, and mix well to make G6P working solution.

Note This G6P working solution is enough for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of G6P standards and test samples in a solid black 96-well microplate. G6P=G6P Standards (G6P1 - G6P7, 0.14 to 100 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
G6P1	G6P1	...	...
G6P2	G6P2	...	...
G6P3	G6P3		
G6P4	G6P4		
G6P5	G6P5		
G6P6	G6P6		
G6P7	G6P7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
G6P1 - G6P7	50 µL	Serial Dilution (0.14 to 100 µM)
BL	50 µL	Dilution Buffer
TS	50 µL	test sample

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1. Prepare G6P standards (G6P), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of G6P working solution to each well of G6P standard, blank control, and test samples to make the total G6P assay volume of 100 µL/well. For a 384-well plate, add 25 µL of G6P working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, Cutoff = 570nm).

Note The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the ratio of A_{575nm}/A_{605nm}. The absorption detection has lower sensitivity compared to fluorescence reading.

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 Glucose-6-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

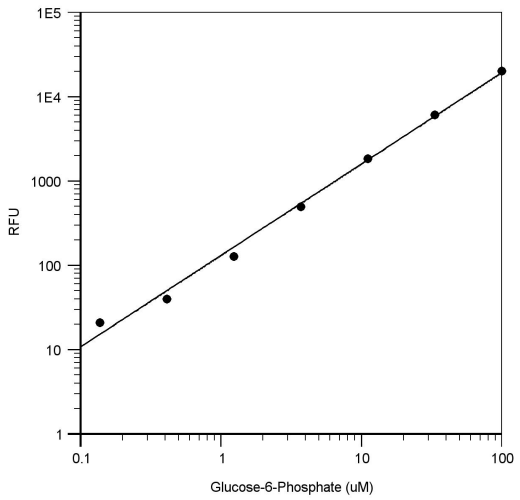

Figure 1. G6P dose response was measured with Amplite™ Fluorimetric G6P Assay Kit in a 96-well solid black plate using a Gemini (Molecular Devices) microplate reader.

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