Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit

Catalog number: 13806
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: G6PD Standard	Freeze (< -15 °C), Minimize light exposure	10 U/vial

OVERVIEW

Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, the first and rate-limiting step in the pentose phosphate pathway. It is critical metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH), and for the production of pentose sugars. The production of NADPH is of great importance for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. The NADPH also maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Deficiencies in G6PD predispose individuals to non-immune hemolytic anemia. AAT Bioquest's Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit provides a simple, sensitive and rapid fluorescence-based method for detecting G6PD in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, G6PD activity is proportionally related to the concentration of NADPH that is specifically monitored by a fluorogenic NADPH sensor to yield a highly red fluorescence product. The fluorescence signal can be read with a fluorescence microplate reader. With the G6PD assay kit, we were able to detect as little as 1 mU/ml G6PD in a 100 µL reaction volume.

AT A GLANCE

Protocol Summary

1. Prepare G6PD standards or test samples (50 µL)
2. Add G6PD working solution (50 µL)
3. Incubate at RT for 30 minutes - 2 hours
4. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important Thaw each kit component at room temperature before starting the experiment.

KEY PARAMETERS

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. NADP stock solution (100X)
Add 100 µL of H_2O into the vial of NADP (Component C) to make 100X NADP stock solution.

2. G6PD standard solution (100 U/mL)

Add 100 µL of H_2O or 1X PBS buffer into the vial of G6PD Standard (Component D) to make 100 U/mL G6PD standard solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13806

G6PD standard
Add 10 µL of G6PD standard solution into 990 µL 1X PBS buffer to generate 1000 mU/mL G6PD standard solution. Take 15 µL of 1000 mU/mL G6PD standard solution into 485 uL of 1X PBS buffer to generate 30 mU/mL G6PD (G6PD7), and then perform 1:3 serial dilutions to get serial dilutions of G6PD standard (G6PD6 - G6PD1). Note: Diluted G6PD standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Probe (Component A). Add 50 µL NADP stock solution (100X) into the bottle of Component A, and mix well.

Note This G6PD assay working solution is enough for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of G6PD standards and test samples in a solid black 96-well microplate. G6PD = D-Glucose-6-Phosphate Dehydrogenase standard (G6PD1 - G6PD7, 0.04 to 30 mU/mL); BL = blank control; TS = test samples.

BL	BL	TS	TS
G6PD1	G6PD1	...	...
G6PD2	G6PD2	...	...
G6PD3	G6PD3		
G6PD4	G6PD4		
G6PD5	G6PD5		
G6PD6	G6PD6		
G6PD7	G6PD7		

Table 2. Reagent Composition for each well.

Well	Volume	Reagent
G6PD1 - G6PD7	50 µL	Serial Dilution (0.04 to 30 mU/mL)
BL	50 µL	Dilution Buffer (PBS)
TS	50 µL	Test Sample

1. Prepare G6PD standards (G6PD), blank controls (BL), and test samples (TS) 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.

2. Add 50 µL of G6PD working solution to each well of G6PD standard, blank control, and test samples to make the total 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.

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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 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 Glucose-6-Phosphate Dehydrogenase 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

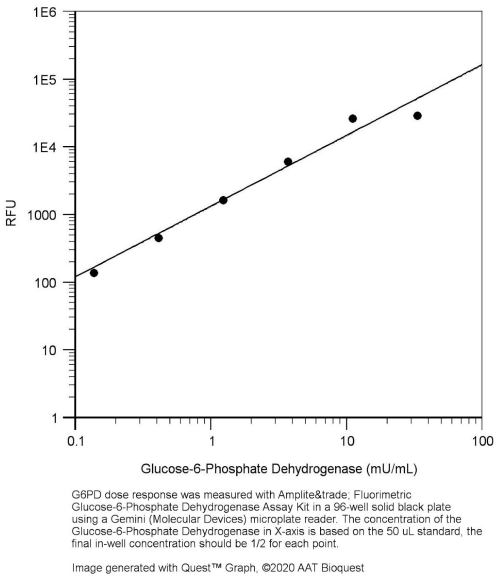

Figure 1. G6PD dose response was measured with Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit in a 96-well solid black plate using a Gemini (Molecular Devices) microplate reader. The concentration of the Glucose-6-Phosphate Dehydrogenase in X-axis is based on the 50 uL standard, the final in-well concentration should be 1/2 for each point.

DISCLAIMER

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