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

Catalog number: 13807
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 G6PDH predispose individuals to non-immune hemolytic anemia. AAT Bioquest's Amplite™ Colorimetric 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 chromogenic NADPH sensor. The absorption signal can be read by an absorption microplate reader at an absorbance ratio of A575 nm to A605 nm. With the G6PD assay kit, we were able to detect as little as 3 mU/ml G6PD in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare G6PD working solution (50 µL)
2. Add G6PD standards or test samples (50 µL)
3. Incubate at room temperature for 30 minutes - 2 hours
4. Monitor absorbance ratio increase at A_{575nm}/A_{605nm}

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

KEY PARAMETERS

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. 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

G6PD standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13807

Add 10 µL of 100 U/mL G6PD standard solution into 990 µL 1x PBS buffer to generate 1000 mU/mL G6PD standard solution. Take 1000 mU/mL G6PD standard solution and perform 1:3 serial dilutions in 1x PBS buffer to get serially diluted G6PD standards (G6PD7 - G6PD1).

Note Diluted G6PD 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 G6PD working solution.

Note This G6PD working solution is enough for one 96-well plate. It is unstable at room temperature and should be used promptly within 2 hours. Avoid exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of G6PD standards and test samples in a white clear bottom 96-well microplate. G6PD=D-Glucose-6-Phosphate Dehydrogenase Standards (G6PD1 - G6PD7, 0.4 to 300 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 Dilutions (0.4 to 300 mU/mL)
BL	50 µL	Dilution Buffer
TS	50 µL	test sample

1. Prepare G6PD standards (G6PD), 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 G6PD working solution to each well of G6PD standard, blank control, and test samples to make the total G6PD assay volume of 100 µL/well. For a 384-well plate, add 25 µL of G6PD 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 absorbance ratio increase with an absorbance plate reader at A_{575nm}/A_{605nm}.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs 575/ Abs 605) 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

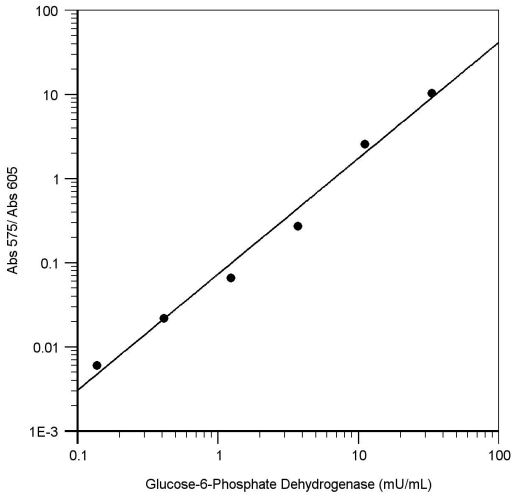

Figure 1. G6PD dose response was measured with Amplite™ Colorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit in a white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader.

DISCLAIMER