

## Amplite™ Colorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13807 (200 assays)	Keep in freezer. Avoid exposure to light	Absorbance microplate readers

### Introduction

Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono- $\delta$ -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™ 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 fluorogenic NADPH sensor to yield a highly red fluorescence product. The fluorescence signal can be read with an absorption microplate reader at the absorbance ratio of  $A_{575nm}/A_{605nm}$ . With the G6PD assay kit, we were able to detect as little as 3mU/ml G6PD in a 100  $\mu$ L reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of G6PD.

### Kit Components

Components	Amount
Component A: Enzyme Probe	2 bottles (lyophilized powder)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: NADP	1 vial
Component D: G6PD Standard	10U/vial

### Assay Protocol for One 96-Well Plate

#### Brief Summary

**Prepare G6PD assay mixture (50  $\mu$ L) → Add G6PD standards or test samples (50  $\mu$ L) → Incubate at room temperature for 30 minutes ~ 2 hours → Monitor absorbance ratio increase at  $A_{575nm}/A_{605nm}$**

*Note: Thaw each kit component at room temperature before starting the experiment.*

#### 1. Prepare NADP stock solution (100X):

Add 100  $\mu$ L of H<sub>2</sub>O into the vial of NADP (Component C) to make 100X NADP stock solution.

#### 2. Prepare G6PD stock solution:

Add 100  $\mu$ L of H<sub>2</sub>O or PBS buffer into the vial of G6PD Standard (Component D) to make 100 U/mL G6PD standard solution.

*Note: The unused G6PD standard stock solution should be divided into single use aliquots and stored at -20°C.*

#### 3. Prepare serial dilutions of G6PD standard (0 to 300 mU/mL):

3.1 Add 10  $\mu$ L of G6PD stock solution (from Step 2) into 990  $\mu$ L PBS buffer to generate 1000 mU/ml G6PD standard solution.

*Note: Diluted G6PD standard solution is unstable, and should be used within 4 hours.*

3.2 Take 200  $\mu$ L of 1000mU/ml G6PD standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3, and 0 mU/mL serial dilutions of G6PD standard.

3.3 Add serial dilutions of G6PD standard and G6PD containing test samples into a white clear bottom 96-well microplate as described in Tables 1 and 2.

**Table 1:** Layout of G6PD standards and test samples in a white clear bottom 96-well microplate

BL	BL	TS	TS	....	....						
G6PD 1	G6PD 1	....	....	....	....						
G6PD 2	G6PD 2										

G6PD 3	G6PD 3										
G6PD 4	G6PD 4										
G6PD 5	G6PD 5										
G6PD 6	G6PD 6										
G6PD 7	G6PD 7										

Note: G6PD = D-Glucose-6-Phosphate Dehydrogenase Standards, BL=Blank Control, TS=Test Samples.

**Table 2** Reagent composition for each well

G6PD Standard	Blank Control	Test Sample
Serial Dilutions*: 50 $\mu$ L	Dilution Buffer : 50 $\mu$ L	50 $\mu$ L

Note: Add the serially diluted G6PD standards from 0.3 mU/mL to 300 mU/mL into wells from G6PD1 to G6PD7 in duplicate.

#### 4. Prepare G6PD assay mixture:

4.1 Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Probe (Component A).

4.2 Add 50  $\mu$ L NADP stock solution (100X) into the bottle of Component A (from Step 4.1), and mix well.

Note: This G6PD assay mixture is enough for one 96-well plate. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.

#### 5. Run G6PD assay:

5.1 Add 50  $\mu$ L of G6PD assay mixture (from Step 4.2) to each well of G6PD standard, blank control, and test samples (see Step 3.3) to make the total assay volume of 100  $\mu$ L/well.

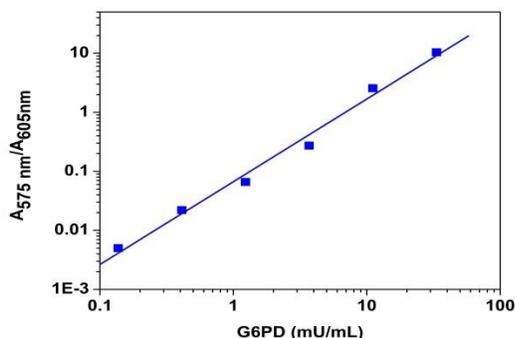
Note: For a 384-well plate, add 25  $\mu$ L of sample and 25  $\mu$ L assay mixture into each well.

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

5.3 Monitor the absorbance ratio increase with an absorbance plate reader at  $A_{575nm}/A_{605nm}$ .

### Data Analysis

For absorbance ratio analysis, one should calculate the ratio in each well first, this will correct the deviation from experiment, such as volume difference in each well. And then subtract the background (with the dilution buffer only) and plot the calibration curve. Usually for the enzyme reaction, the Log-Log fit will give a linear curve. And then use the Equation to calculate the concentration of the sample.



**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. As low as 0.3 mU/mL glucose-6-phosphate dehydrogenase in 100  $\mu$ L volume can be detected with 1 hour incubation.

### References

1. Corpas FJ, Barroso JB, Sandalio LM, Distefano S, Palma JM, Lupiáñez JA, Del Río LA (March 1998). "A dehydrogenase-mediated recycling system of NADPH in plant peroxisomes". *Biochem. J.* 330 ( Pt 2) : 777–84.
2. Gaskin RS, Estwick D, Peddi R (2001). "G6PD deficiency: its role in the high prevalence of hypertension and diabetes mellitus". *Ethnicity & disease* 11 (4): 749–54.
3. Wajcman H, Galactéros F (2004). "[Glucose 6-phosphate dehydrogenase deficiency: a protection against malaria and a risk for hemolytic accidents]". *C. R. Biol.* 327 (8): 711–20.
4. Frank JE (October 2005). "Diagnosis and management of G6PD deficiency". *Am Fam Physician* 72 (7): 1277–82.
5. Aster J, Kumar V, Robbins SL, Abbas AK, Fausto N, Cotran RS (2010). *Robbins and Cotran pathologic basis of disease*.

**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®, Inc.. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**