Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13804 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

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 at Ex/Em = 530-570 nm/590-600 nm (Ex/Em = 540 nm/590 nm is recommended). With the Amplite™ G6P Assay Kit, we were able to detect as little as 0.3 µM G6P in a 100 µL reaction volume.

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: Glucose-6-Phosphate Standard	3.04mg/vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare G6P assay mixture (50 µL) → Add G6P standards or test samples (50 µL) → Incubate at room temperature for 30 minutes ~ 2 hours → Monitor fluorescence increase at Ex/Em = 540/590 nm

Note: Thaw kit components at room temperature before starting the experiment.

1. Prepare NADP stock solution (100X):

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

2. Prepare G6P stock solution:

Add 100 µL of H_2O or 1xPBS buffer into the vial of G6P Standard (Component D) to make 100 mM G6P standard solution.
Note: The unused G6P standard stock solution should be divided into single use aliquots and stored in a freezer.

3. Prepare serial dilutions of G6P standard (0 to 100 µM):

3.1 Add 10 µL of G6P stock solution (from Step 2) into 990 µL 1x PBS buffer to generate 1 mM G6P standard solution.
And then add 10 µL of 1mM G6P stock solution into 990 µL 1x PBS buffer to generate 100 µM G6P standard solution.
Note: Diluted G6P standard solution is unstable, and should be used within 4 hours.

3.2 Take 200 µL of 1 mM G6P standard solution to perform 1:3 serial dilutions to get 30, 10, 3, 1, 0.3, 0.1 and 0 µM serial dilutions of G6P standard.

3.3 Add serial dilutions of G6P standard and G6P containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1: Layout of G6P standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
G6P 1	G6P 1	….	….	….	….						
G6P 2	G6P 2										
G6P 3	G6P 3										
G6P 4	G6P 4										
G6P 5	G6P 5										

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G6P 6	G6P 6										
G6P 7	G6P 7										

Note: G6P=G6P Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

G6P Standard	Blank Control	Test Sample
Serial Dilutions*: 50 µL	Dilution Buffer : 50 µL	50 µL

**Note: Add the serially diluted G6P standards from 0.1 µM to 100 µM into wells from G6P1 to G6P7 in duplicate.*

4. Prepare G6P assay mixture:

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

4.2 Add 50 µL NADP stock solution (100X, from Step 1) into the bottle of Component A (from Step 4.1), and mix well.
Note: This G6P assay mixture is enough for one 96-well plate. The unused assay mixture should be divided into single use aliquots and stored at -20°C.

5. Run G6P assay:

5.1 Add 50 µL of G6P assay mixture (from Step 4.2) to each well of G6P standard, blank control, and test samples (see Step 3.3) to make the total assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL 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 fluorescence increase with a fluorescence plate reader at Ex/Em = 530-570/590-600 nm (optimal Ex/Em = 540/590 nm, cut off at 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.

Data Analysis

The fluorescence in blank wells (with the dilution buffer only) is used as a control, and is subtracted from the values for those wells with the G6P reactions. A typical G6P standard curve is shown in Figure 1.

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

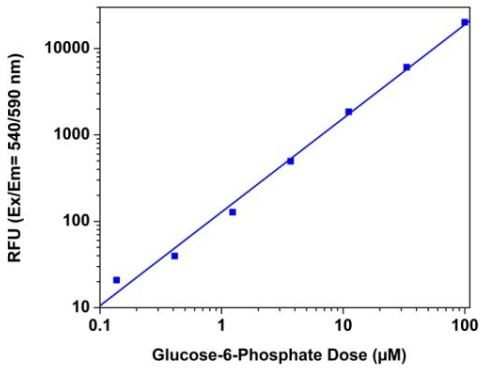

Figure 1. G6P dose response was measured with Amplite™ Fluorimetric G6P Assay Kit in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 0.3µM G6P in 100 µL volume can be detected with 1 hour incubation.

References

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