Glucose-6-phosphate Dehydrogenase Assay (G6PDH)

Cat. No. 8428
100 Tests in 96-well plate

Introduction

Glucose-6-phosphate dehydrogenase (G6PDH) is a cytosolic enzyme that catalyzes the first step in the pentose phosphate pathway (PPP). The PPP pathway is critical for maintaining the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) and for the production of ribose-5-phosphate. G6PDH deficiency, the most common enzyme deficiency worldwide, predisposes individuals to non-immune hemolytic anemia. This colorimetric assay is based on glucose-6-phosphate dehydrogenase catalyzed oxidation of glucose-6-phosphate, in which the formed NADPH can convert a nearly colorless probe to an intensely colored product, which exhibits maximum absorbance at 440nm, is proportional to the amount of G6PDH in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8428a	1	Assay buffer	10 mL	4°C
8428b	1	G6PDH standard	0.1 mL	-20°C
8428c	1	Developer (10X)	0.1 mL	-20°C
8428d	1	NADP	0.6 mL	-20°C
8428e	1	WST	3.91 mg	-20°C
8428f	1	Substrate	0.5 mL	-20°C

Product Use

Glucose-6-phosphate Dehydrogenase Assay kit could measure G6PDH activity of samples from cells and tissue. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from glucose-6-phosphate dehydrogenase assay of G6PDH solutions with concentrations ranging from 0.02 to 1 U/ml show a linear relationship between OD_{440nm} and G6PDH activity (Figure 1).

Shipping

Shipped on dry ice.

Sample Preparation

1. Developer solution (1X): dilute developer (10X) (8428c) in assay buffer (8428a) (1:10).
2. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8428a). Vortex briefly and keep in the dark at -20°C until use. For longer storage, we suggest that you aliquot and store the reconstituted WST solution at -20°C, avoid repeated freeze/thaw cycles.

Procedure (96-well plate)

A. Preparation of glucose-6-phosphate dehydrogenase standard

1. Add 1 µL of G6PDH standard (8428b) to 99 µL of assay buffer (8428a) to make a 0.1 mL solution of 2 U/mL G6PDH.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8428a) into each tube and label them #1 through #7.
3. Add 25 µL of the 2 U/mL into tube #1 and mix well to get the 1U/mL G6PDH standard.
4. Transfer 25 µL of the 1U/mL G6PDH standards from tube #1 to tube #2 and mix well to get the 0.5U/mL G6PDH standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the G6PDH standards. Do not add any G6PDH to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each G6PDH standard by aliquoting 10 µL/well of each G6PDH standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	1 U/ml	0.5 U/ml	0.25 U/ml	0.125 U/ml	0.0625 U/ml	0.03125 U/ml	Blank
B	1 U/ml	0.5 U/ml	0.25 U/ml	0.125 U/ml	0.0625 U/ml	0.03125 U/ml	Blank

B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8428a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous NADH or NADPH from cell or tissue extracts would generate background for the G6PDH assay. To remove the NADH or NADPH background, same amount of sample can be tested in the absence of substrate.
3. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 70 µL assay buffer (8428a), 5 µL developer solution (1X), 5 µL NADP (8428d) and 5 µL WST solution, 5 µL substrate(8428f). Without adding substrate into the well containing test samples for control.
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing G6PDH standard, test samples, blank and test samples for control. Incubate for 30 minutes at room temperature in dark.
3. Read the absorbance at 440 nm with an ELISA plate reader.

D. Calculations

1. Average the OD_{440nm} of duplicate wells of each G6PDH standard, test sample and blank. Subtract the OD_{440nm} value of the blank from the OD_{440nm} values obtained with all other standard and samples to get ΔOD_{440nm} value.
2. Based on the calibrated ΔOD_{440nm} of the G6PDH standard, make a standard curve by plotting ΔOD_{440nm} as a function of G6PDH activity (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. For samples requiring control without substrate, subtract the ΔOD_{440nm} without substrate value from the ΔOD_{440nm} with substrate value and use this $\Delta\Delta OD_{440nm}$ value to determine the sample G6PDH activity from the standard curve.
4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the G6PDH activity of test samples as follows:

$$[G6PDH] = \frac{\Delta\Delta\ OD_{440nm}}{A}$$

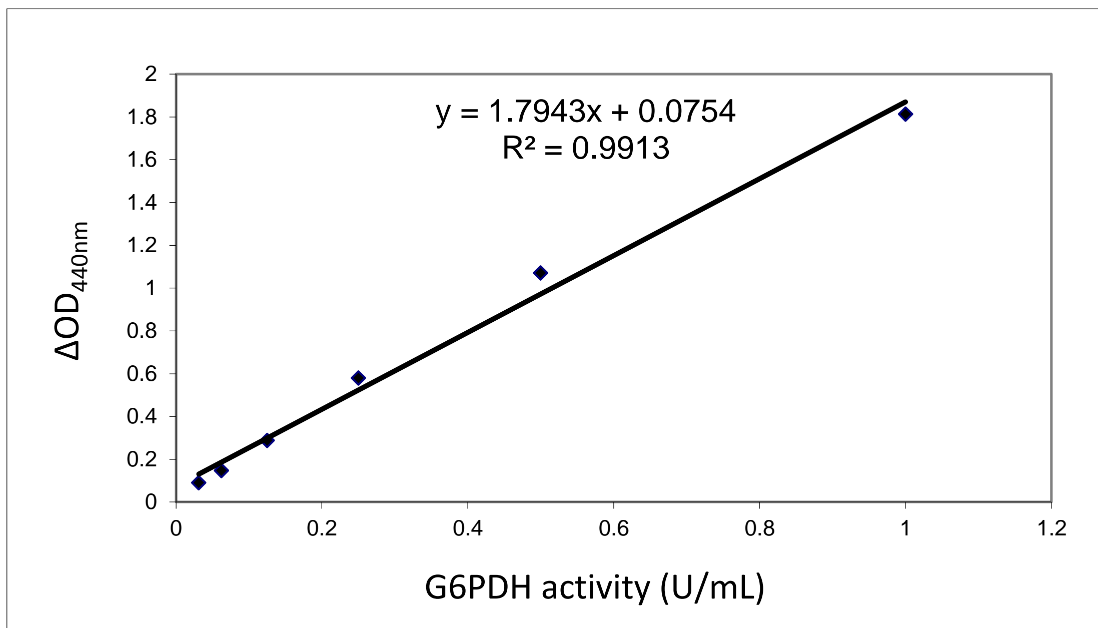

Figure1. A typical G6PDH standard curve measured by ScienCell™ Glucose-6-phosphate Dehydrogenase Assay kit