Glucose-6-phosphate Assay (G6P)

Cat. No. 8398
100 Tests in 96-well plate

Introduction

Glucose-6-phosphate (G6P) is the first substrate in glycolysis, the hexose monophosphate shunt and both the glycogen and starch biosynthesis pathways. G6P is utilized by glucose-6-phosphate dehydrogenase to generate reducing equivalents in the form of NAD(P)H. This is particularly important in red blood cells where a G6PDH deficiency leads to hemolytic anemia. This colorimetric assay is based on glucose-6-phosphate dehydrogenase catalyzed oxidation of G6P, 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 G6P in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8398a	1	Assay buffer	10 mL	4°C
8398b	1	G6P standard	0.1 mL	-20°C
8398c	1	Substrate (10X)	0.1 mL	-20°C
8398d	1	NADP	0.6 mL	-20°C
8398e	1	WST	3.91 mg	-20°C
8398f	1	Enzyme (100X)	20 µL	-20°C

Product Use

Glucose-6-phosphate Assay kit could measure G6P level 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 assay of G6P solutions with concentrations ranging from 1.0 to 500 µM show a linear relationship between OD_{440nm} and G6P concentration (Figure 1).

Shipping

The kit would be shipped on dry ice.

Sample Preparation

1. Substrate solution (1X): dilute substrate (10X) (8398c) in assay buffer (8398a) (1:10).
2. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8398a). 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.
3. Enzyme (1X): dilute enzyme (100X) (8398f) in assay buffer (8398a) (1:100).

Procedure (96-well plate)

A. Preparation of glucose-6-phosphate standard

1. Add 2 µL of G6P standard (8398b) to 98 µL of assay buffer (8398a) to make a 0.1 mL solution of 400 µM G6P.
2. Obtain 7 test tubes, add 70 µL of assay buffer (8398a) into each tube and label them #1 through #7.
3. Add 70 µL of the 400 µM into tube #1 and mix well to get the 200 µM G6P standard.
4. Transfer 70 µL of the 200 µM G6P standards from tube #1 to tube #2 and mix well to get the 100 µM G6P standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the G6P standards. Do not add any G6P to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each G6P standard by aliquoting 30 µL/well of each G6P standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	200 µM	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	Blank
B	200 µM	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	Blank

B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8398a). 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 G6P assay. To remove the NADH or NADPH background, same amount of sample can be tested in the absence of enzyme (1X).
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 30 µL/well on the 96-well flat bottom plate.

Recommendation:

Endogenous enzyme may degrade G6P quickly. Samples (such as cell or tissue lysate) should be deproteinized with a 10k Da MW spin filter (Millipore UFC501008) or 0.5M metaphosphoric acid (Sigma 239275) and kept at -80°C for storage.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 50 µL assay buffer (8398a), 5 µL substrate, (1X), 5 µL NADP (8398d) and 5 µL WST solution, 5 µL enzyme (1X). Without adding enzyme (1X) into the well containing test samples for control.
2. Add 70 µL of working reagent mix into each well of the 96-well plate containing G6P 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 G6P 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 G6P standard, make a standard curve by plotting ΔOD_{440nm} as a function of G6P concentration (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 enzyme, subtract the ΔOD_{440nm} without enzyme value from the ΔOD_{440nm} with enzyme value and use this $\Delta\Delta OD_{440nm}$ value to determine the sample G6P concentration from the standard curve.
4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the G6P concentration of test samples as follows:

$$[G6P] = \frac{\Delta\Delta\ OD_{440nm}}{A}$$

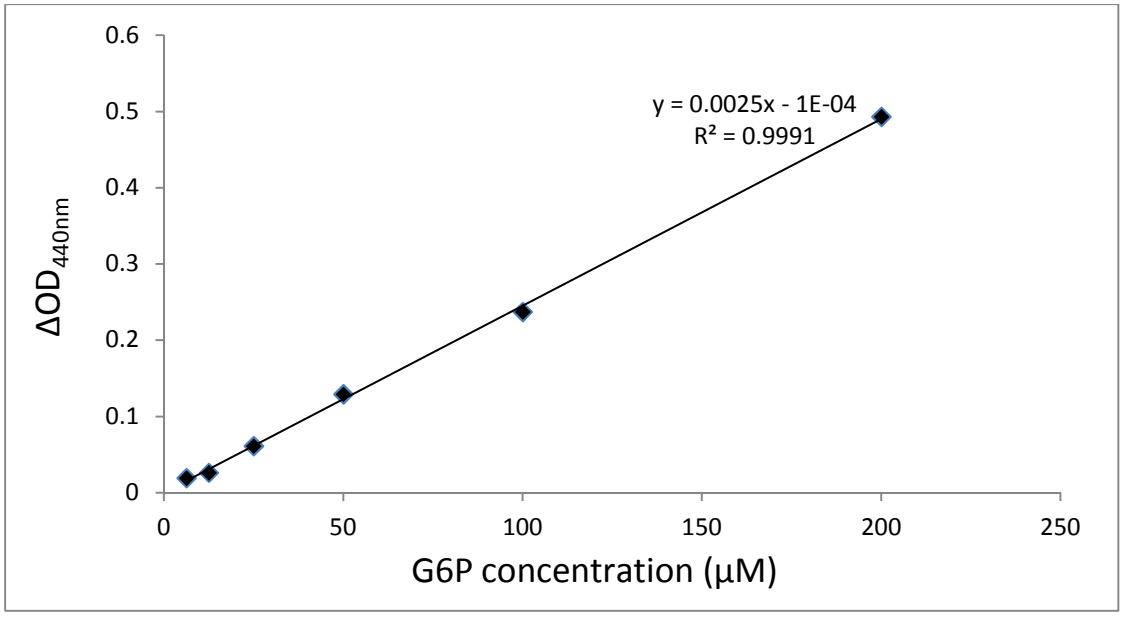

Figure1. A typical G6P standard curve measured by ScienCell™ Glucose-6-phosphate Assay kit