QuantiChrom™ Glucose-6-Phosphate Dehydrogenase Kit (DGPDH-100)

Quantitative Colorimetric Kinetic Glucose-6-Phosphate Dehydrogenase Activity Determination

DESCRIPTION

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH) is a cytosolic enzyme in the pentose phosphate pathway which supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). G6PDH reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate (G6P). Humans with a genetic deficiency of G6PDH are predisposed to non-immune hemolytic anemia. BioAssay Systems' non-radioactive, colorimetric G6PDH assay is based on the reduction of the tetrazolium salt MTT in a NADPH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.2 to 100 U/L for 15 min reaction.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

G6PDH activity determination in biological samples (e.g. plasma, serum, erythrocytes, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Diaphorase:**	120 µL
NADP/MTT:	1 mL	**Calibrator:**	1.5 mL
Substrate:	1 mL		

Storage conditions. The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all assay wells by mixing, for each 96-well assay: 8 µL Substrate, 8 µL NADP/MTT Solution, 1 µL Diaphorase and 70 µL Assay Buffer.

Reaction Preparation:

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells and then add 80 µL WR to each sample well. Tap plate briefly to mix.
3. Read OD_{565nm} (OD_0), and again after 15 min (OD_{15}) on a plate reader.

CALCULATION

Subtract the OD_0 from OD_{15} for each sample to compute the ΔOD_S values. G6PDH activity can then be calculated as follows:

$$\text{G6PDH Activity} = \frac{\Delta OD_S}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S}{OD_{CAL} - OD_{H20}} \times \frac{273}{t\,\text{(min)}} \times n \quad \textit{(U/L)}$$

where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_o) values of the Calibrator and water. t is the reaction time (15 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

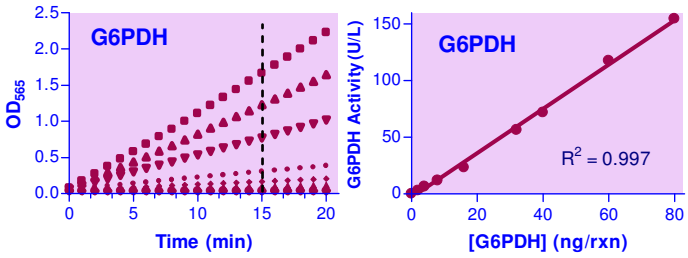
Unit definition: 1 Unit (U) of G6PDH will catalyze the conversion of 1 µmole of NADP to NADPH per min at pH 8.2.

Note: If sample G6PDH activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with G6PDH activity < 1 U/L, the incubation time can be extended to 2 hours.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

G6PDH Titration Curves



Raw Kinetic Data ***G6PDH Activity (15 min, 25 °C)***

LITERATURE

1. Glock, GE and McLean, P (1953) Further Studies on the Properties and Assay of Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase of Rat Liver. Biochem. 55:400-8.
2. Kirman, HN and Hendrickson, EM (1962) Glucose 6-Phosphate Dehydrogenase from Human Erythrocytes II. Subactive states of the enzyme from normal persons. J. Biol. Chem. 237: 2371-6.
3. Tian, W-N et. al. (1998) Importance of Glucose-6-phosphate Dehydrogenase Activity for Cell Growth. J. Biol. Chem. 273: 10609-17.