Glucose 6 phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

Glucose 6 phosphate Dehydrogenase Activity Assay Kit (Colorimetric) can be used to measure Glucose 6 phosphate Dehydrogenase activity in serum, plasma, tissue and cell culture supernatants.

Catalog number: ARG82165

Package: 100 tests

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD or G6PDH) (EC 1.1.1.49) is a cytosolic enzyme that catalyzes the chemical reaction:

D-glucose 6-phosphate + $NADP^+$ + H_2O ⇌ 6-phospho-D-glucono-1, 5-lactone + NADPH + H^+

This enzyme participates in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage from compounds like hydrogen peroxide. Of greater quantitative importance is the production of NADPH for tissues involved in biosynthesis of fatty acids or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. G6PD reduces $NADP^+$ to NADPH while oxidizing glucose-6-phosphate. [Provide by Wikipedia: Glucose-6-phosphate dehydrogenase]

PRINCIPLE OF THE ASSAY

This Glucose 6 phosphate Dehydrogenase Activity Assay Kit (Colorimetric) is a simple assay that measures the amount of glucose 6 phosphate dehydrogenase (G6PDH) present in biological samples. This 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 O.D. 565 nm. The increase in absorbance at O.D. 565 nm is proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Diaphorase	120 µL	-20°C
NADP/MTT	1 mL	-20°C
Substrate	1 mL	-20°C
Standard	1.5 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well microplate
- Deionized or distilled water
- Pipettes, pipette tips and multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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).
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Cell or tissue lysate: Collect cell or tissue into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of 1X PBS for 5×10^6 cell or 0.05 g tissue, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 15 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Note:

- All sample can be stored at-80 to -20°C for at least one month.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mixing 70 µL of Assay Buffer, 8 µL of Substrate, 8 µL of NADP/MTT and 1 µL of Diaphorase. Prepare immediately before assay.

ASSAY PROCEDURE

Equilibrate Substrate Buffer to desired reaction temperature (E.g., 25°C or 37°C). Briefly centrifuge tubes before use.

	Standard well	Sample well	H_2O well
Standard	100 µL		
Distilled water			100 µL
Samples		20 µL	
Working Reagent		80 µL	
Tap plate to mix briefly and thoroughly. Incubate for **0** and **15 minutes** at **room temperature**.			
Read the absorbance at **O.D. 565 nm**. (**OD_0 and OD_{15}**)			

CALCULATION OF RESULTS

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

   G6PDH Activity (U/L)

   = $[\Delta OD_S / (\varepsilon_{mtt} \times l)] \times [\text{Reaction Vol} / (t \times \text{Sample Vol})] \times n$

   = $(273 / t) \times [\Delta OD_S / (OD_{STANDARD} - OD_{H2O})] \times n$

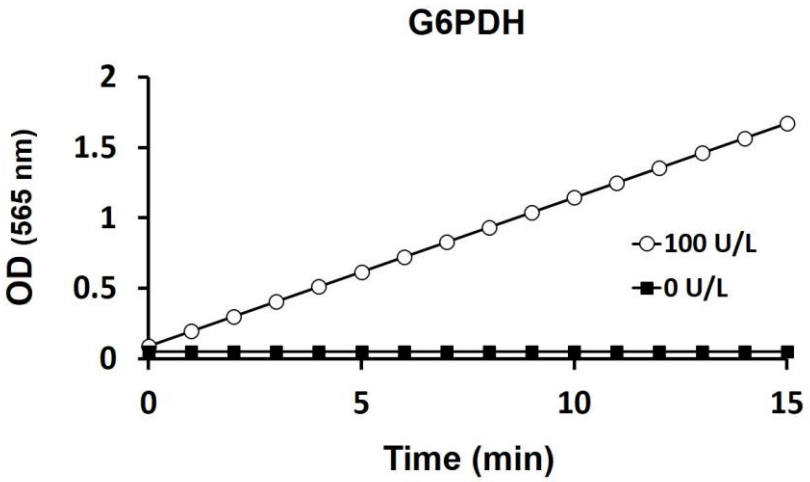
   Note:

   - ε_{mtt}: the molar absorption coefficient of reduced MTT.
   - *l*: the light path length which is calculated from the calibrator.
   - $OD_{STANDARD}$ and OD_{H2O}: the O.D. 565 nm (OD_0) value of Standard and H_2O wells.
   - t: the reaction time (15 minutes is the recommended time).
   - Reaction Vol and Sample Vol: 100 µL and 20 µL, respectively.
   - n: the dilution factor if the sample needed to be diluted.

2. If sample G6PDH activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in distilled water and repeat the assay. For samples with G6PDH activity < 1 U/L, the incubation time can be extended up to 2 hours.
3. Unit definition: 1 Unit (U) of G6PDH will catalyze the conversion of 1 µmole of NADP to NADPH per min at pH 8.2.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glucose 6 phosphate Dehydrogenase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.2 U/L