Glucose 6 phosphate Assay Kit (Colorimetric)

Glucose 6 phosphate Assay Kit (Colorimetric) is a detection kit for the quantification of Glucose 6 phosphate in serum, plasma, tissue and cell culture supernatants.

Catalog number: ARG82164

Package: 100 tests

TABLE OF CONTENTS

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INTRODUCTION

Glucose 6-phosphate (G6P, sometimes called the Robison ester) is a glucose sugar phosphorylated at the hydroxy group on carbon 6. This dianion is very common in cells as the majority of glucose entering a cell will become phosphorylated in this way.

Because of its prominent position in cellular chemistry, glucose 6-phosphate has many possible fates within the cell. It lies at the start of two major metabolic pathways: glycolysis and the pentose phosphate pathway.

In addition to these two metabolic pathways, glucose 6-phosphate may also be converted to glycogen or starch for storage. This storage is in the liver and muscles in the form of glycogen for most multicellular animals, and in intracellular starch or glycogen granules for most other organisms. [Provide by Wikipedia: Glucose 6 phosphate]

PRINCIPLE OF THE ASSAY

This Glucose 6 phosphate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Glucose 6 phosphate (G6P) present in biological samples. G6P is oxidized by glucose-6-phosphate dehydrogenase and the formed NADPH is coupled to the formazan (WST-8) chromogen. The intensity of the product color, measured at O.D. 460 nm, is proportional to the G6P concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
NADP/WST8	1 mL	-20°C
Standard (100 mM G6P)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 460 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.
- 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.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- 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 2×10^6 cell or 0.1 g tissue, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Note:

1. Serum and plasma samples can be measured directly but may need a sample blank if they have significant absorbance at O.D. 460 nm.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mix 75 µL of Assay Buffer, 8 µL of NADP/WST8, 1 µL of Enzyme A and 1 µL of Enzyme B. Prepare immediately before assay. If including Sample Blanks, prepare a Blank Working Reagent (BWR) without the Enzyme A.
- **Standards:** mix 5 µL of 100 mM Standard with 495 µL of distilled water (final 1000 µM). Dilute standards as follows.

Standard tube	G6P (µM)	Distilled water (µL)	Standard Premix, 1000 µM (µL)
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep reconstituted Enzyme Mix tube on ice during assay.

	Standard well	Sample well	Blank well
Standard	20 µL		
Sample		20 µL	20 µL
Working Reagent	80 µL	80 µL	
Blank Working Reagent			80 µL
Tap plate to mix briefly and thoroughly. Incubate for **20 minutes** at **room temperature** in the dark.			
Read the absorbance at **O.D. 460 nm**.			

Note:

- If the calculated G6P concentration is >1000 µM, dilute sample in distilled water and repeat assay. Multiply result by the dilution factor.
- For samples that may have background absorbance at O.D. 460 nm or significant levels of NADH or NADPH (> 20 µM), add 20 µL of the sample to a second well to serve as a sample blank.

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the G6P concentration of Sample as follows:

   G6P (µM) = [(OD_{Sample} – OD_{Blank}) / Slope (μM^{-1})] x n

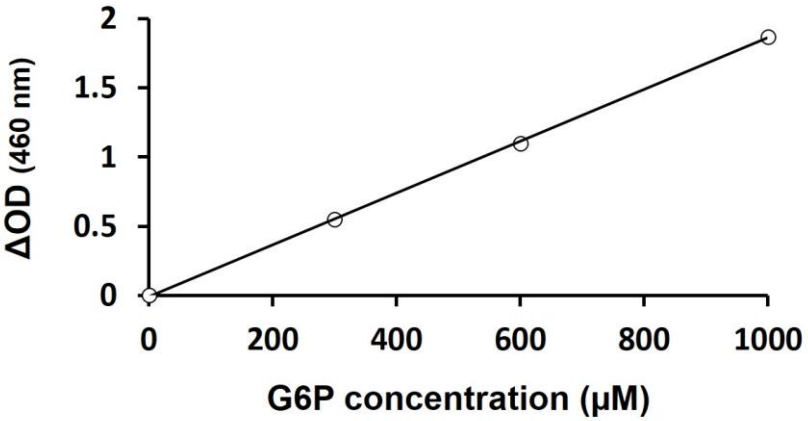
   Note:

   - OD_{Sample}, OD_{Blank}: the O.D. 460 nm values of the sample and distilled water blank or sample blank.
   - n is the sample dilution factor.

2. If the calculated G6P concentration is >1000 µM, dilute sample in distilled water and repeat assay. Multiply result by the dilution factor.
3. Conversions: 100 µM G6P equals 34 mg/L, 0.0034% or 34 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glucose 6 phosphate 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

10 µM