

Glucose-6-Phosphate (G6P) Assay Kit (Colorimetric)

LS-K279-100 (100 Tests) • Store at -20°C



Introduction

GLUCOSE-6-PHOSPHATE (G6P) is glucose sugar phosphorylated on carbon 6. Most of the glucose entering cells is phosphorylated to G6P. G6P has three primary fates within the cell. It lies at the start of two major metabolic pathways: glycolysis and the pentose phosphate pathway. In addition to these metabolic pathways, glucose 6-phosphate may also be converted to glycogen or starch for storage. LSBio's Glucose- 6-Phosphate Assay Kit provides a simple, and automation-ready procedure for measuring G6P concentration. 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 460 nm, is proportional to the G6P concentration in the sample.

Key Features

- Fast and sensitive. Linear detection range: 10 to 1000 μ M G6P.
- 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

- G6P determination in biological samples (e.g. plasma, serum, tissue and culture media.)

Components

Component	K279-100
	100 Tests
Assay Buffer	10 mL
NADP/WST9	1 mL
Enzyme A	120 μ L
Enzyme B	120 μ L
Standard (100 mM G6P)	100 μ L

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Sample Preparation

Tissue or cell samples (2×10^6) can be homogenized in 100 μ L PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay. Serum and plasma samples can be measured directly but may need a sample blank if they have significant absorbance at 460 nm.

Assay Procedure

- Standards. Dilute the G6P Standard to 1000 μ M Premix by mixing 5 μ L of the 100 mM Standard with 495 μ L dH₂O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the table.

No	Premix + dH ₂ O	G6P (μ M)
1	100 μ L + 0 μ L	1000
2	60 μ L + 40 μ L	600
3	30 μ L + 70 μ L	300
4	0 μ L + 100 μ L	0

Transfer 20 μ L of each standard to separate wells in a 96 well plate.

- Samples. Add 20 μ L of each sample to separate wells in a 96 well plate. (For samples that may have background absorbance at 460 nm or significant levels of NADH or NADPH ($> 20 \mu$ M), add 20 μ L of the sample to a second well to serve as a sample blank).
- G6P Detection. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 75 μ L Assay Buffer, 8 μ L NADP/WST8, 1 μ L Enzyme A and 1 μ L Enzyme B. If including Sample Blanks, prepare a blank working reagent (BWR) without the Enzyme A. Add 80 μ L of the appropriate WR to each Standard and Sample well. Mix well and incubate protected from light for 20 min at RT.
- Read OD_{460nm}.

Calculations

Subtract the blank value (#4) from the standard values and plot the DOD against standard concentrations. Determine the slope and calculate the G6P concentration of the Samples as follows:

$$[\text{G6P}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \times n \text{ (}\mu\text{M)}$$

where OD_{SAMPLE} is the OD reading of the Sample and OD_{BLANK} is the OD reading of the Blank (Standard #4) or Sample Blank if used. n is the sample dilution factor. If the calculated G6P concentration is $>1000 \mu$ M, dilute sample in dH₂O and repeat assay. Multiply result by the dilution factor.

Conversions: 100 μ M G6P equals 34 mg/L, 0.0034% or 34 ppm.

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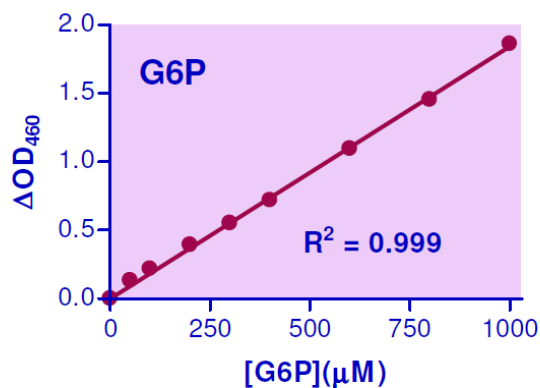
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Sample Data



Standard Curve in 96-well plate assay

Version: V.08.09.2018

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