Technical Bulletin

Glucose-6-Phosphate Assay Kit

Catalogue number MAK503

Product Description

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.

The 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.

The detection range of the kit is 10 to 1000 µM G6P. The kit is used to determine the G6P determination in biological samples such as plasma, serum, tissue, and culture media.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK503A
- Enzyme A 120 µL
  Catalogue Number MAK503B
- Enzyme B 120 µL
  Catalogue Number MAK503C
- NADP/WST8 1 mL
  Catalogue Number MAK503D
- Standard (100 mM G6P) 100 µL
  Catalogue Number MAK503E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5mL microcentrifuge tubes
- Phosphate Buffered Saline (catalogue number PPB006 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Equilibrate all components to room temperature prior to use. Briefly centrifuge small vials prior to opening.

Procedure

Equilibrate all components to room temperature prior to use. Briefly centrifuge small vials prior to opening.

Sample Preparation

Tissue or cell samples (2 x10^6) can be homogenized in 100µL PBS. Centrifuge at 14,000 rpm for 5 minutes. 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.

Standard Curve Preparation

1. Dilute the G6P Standard to 1000 µM Premix by mixing 5 µL of the 100 mM Standard with 495 µL purified water.
2. Further dilute the standards in 1.5 mL centrifuge tubes as described in Table 1.

Table 1.
G6P Standard Dilutions

Well	G6P Standard Premix	Purified Water	G6P (µM)
1	100 µL	-	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	-	100	0

3. Transfer 20 µL of each standard to separate wells in a 96-well plate.

Working Reagent Preparation

Prepare enough Working Reagent for all standards and samples.

Reagent	Volume
Assay Buffer	75 µL
NADP/WST8	8 µL
Enzyme A	1 µL
Enzyme B	1 µL

Note: If including Sample Blanks, prepare a blank working reagent without Enzyme A.

Assay Reaction

1. Add 20 µL of each sample to separate wells in a 96-well plate.

Note: For samples that may have background absorbance at 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.

2. Add 80 µL of the appropriate WR to each Standard and Sample well.
3. Mix well and incubate protected from light for 20 minutes at room temperature.

Measurement

Measure the optical density at 460_{nm}.

Results

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

$$\text{G6P (µM)} = \frac{OD_{Sample} - OD_{Blank}}{\text{Slope}} \times DF$$

Note: If the calculated G6P concentration is >1000 µM, dilute sample in purified water and repeat the assay. Multiply the result by the new dilution factor.

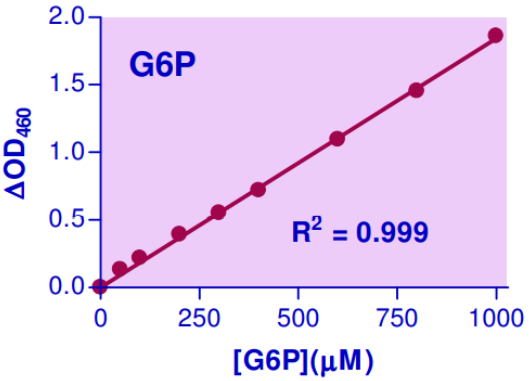
Where:

OD_{SAMPLE} = OD values of the Sample

OD_{BLANK} = OD values of the Blank

DF = Dilution factor
Unit conversion: 100 µM G6P equals 34 mg/L, 0.0034% or 34 ppm.

Figure 1.
Typical G6P Standard Curve



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