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Product Information

Glucose-6-Phosphate Assay Kit

Catalog Number **MAK014**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glucose 6-phosphate (G6P) is a key metabolic intermediate that enters either metabolic pathways or storage. G6P is generated when glucose is phosphorylated by hexokinase or glucokinase, or by the conversion of glucose-1-phosphate by phosphoglucomutase during glycogenolysis. G6P lies at the beginning of both glycolysis and the pentose phosphate pathways. It also can be stored as glycogen when blood glucose levels are high. G6P is utilized by glucose-6-phosphate dehydrogenase (G6PDH) to generate reducing equivalents in the form of NADPH. This is particularly important in red blood cells where a G6PDH deficiency leads to hemolytic anemia.

The Glucose-6-phosphate Assay Kit is a simple, sensitive and rapid means of quantifying G6P in a variety of samples. G6P is determined by an enzyme assay, which results in a colorimetric (450 nm) product, proportional to the G6P present. Typical detection range for this kit is 2–10 nmoles.

Components

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

Glucose-6-Phosphate Assay Buffer Catalog Number MAK014A	25 mL
Glucose-6-Phosphate Enzyme Mix Catalog Number MAK014B	1 vl
Glucose-6-Phosphate Substrate Mix Catalog Number MAK014C	1 vl
Glucose-6-Phosphate Standard, 10 µmole Catalog Number MAK014D	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for serum-containing samples).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glucose-6-Phosphate Assay Buffer – Allow buffer to come to room temperature before use.

Glucose-6-Phosphate Enzyme Mix – Reconstitute in 220 µL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Glucose-6-Phosphate Substrate Mix – Reconstitute in 220 µL of Glucose-6-Phosphate Assay Buffer. Mix well by pipetting, then aliquot and store at 4 °C. Use within 2 months of reconstitution.

Glucose-6-Phosphate Standard – Reconstitute in 100 µL of water to generate a 100 mM Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glucose-6-Phosphate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM G6P Standard Solution with 990 µL of water to generate a 1 mM standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glucose-6-Phosphate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid or solution samples may be assayed directly. If enzymes are present in the samples, samples should be deproteinized with a 10 kDa MWCO spin filter.

Tissue (10–100 mg) or cells (1–5 $\times$ 10^6) can be homogenized in 2–3 volumes of ice cold PBS or other buffer (pH 6.5–8). Centrifuge the samples at 13,000 $\times$ *g* for 10 minutes to remove insoluble material. Samples should be deproteinized with a 10 kDa MWCO spin filter.

Add 1–50 µL samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Glucose-6-Phosphate Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Assay Reaction
1. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well). Note: NADH or NADPH in samples will generate background readings. To remove the effect of NADH or NADPH background, a blank sample may be set up for each sample by omitting the Glucose-6-Phosphate Enzyme Mix. The blank readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Blank Sample	Samples and Standards
G6P Assay Buffer	48 µL	46 µL
G6P Enzyme Mix	–	2 µL
G6P Substrate Mix	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) G6P standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate G6P standards to plot a standard curve. The amount of G6P present in the samples may be determined from the standard curve.
Note: A new standard curve must be set up each time the assay is run.

Concentration of G6P

$$S_a/S_v = C$$

S_a = Amount of G6P in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells.
C = Concentration of G6P in sample

Glucose-6-Phosphate molecular weight: 260.14 g/mole

Sample Calculation
G6P amount (S_a) = 5.84 nmole
Assay volume (S_v) = 50 µL

Concentration of G6P in sample

5.84 nmole/50 µL = 0.1169 nmole/µL

0.1169 nmole/µL × 260.14 ng/nmole= 30.41 ng/µL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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