

Product Information

High Sensitivity Glucose-6-Phosphate Assay Kit

Catalog Number **MAK021**

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 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.

This Glucose-6-phosphate Assay Kit is a simple, sensitive, and rapid means of measuring G6P in a variety of samples. It is much more sensitive than the Glucose-6-Phosphate Colorimetric Assay Kit (MAK014) and is suitable for samples with low concentrations of glucose-6-phosphate. Glucose-6-phosphate concentration is determined by a coupled enzyme assay, which results in a fluorometric ($\lambda_{\text{ex}} = 535 \text{ nm}$ / $\lambda_{\text{em}} = 587 \text{ nm}$) product, proportional to the G6P present. Typical detection range for this kit is between 10–500 pmoles.

Components

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

Glucose-6-Phosphate Assay Buffer Catalog Number MAK021A	25 mL
Glucose-6-Phosphate Probe, in DMSO Catalog Number MAK021B	0.4 mL
Glucose-6-Phosphate Enzyme Mix Catalog Number MAK021C	1 vL
Glucose-6-Phosphate Substrate Mix Catalog Number MAK021D	1 vL
Glucose-6-Phosphate Standard, 10 μmole Catalog Number MAK021E	1 vL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

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 Probe – Warm to room temperature before use to melt DMSO. Mix well by pipetting, then aliquot and store at -20°C .

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

Glucose-6-Phosphate Standard - Reconstitute in 100 μL of water to generate 100 mM standard solution. Mix well by pipetting, then aliquot and store at -20°C . Keep cold while in use. 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.

G6P Standards for Fluorometric Detection

Dilute 10 μL of the 100 mM G6P standard solution with 990 μL of water to prepare a 1 mM standard solution. Take 10 μL of the 1 mM standard solution and add to 990 μL of water to make a 0.01 mM G6P standard solution. Add 0, 1, 2, 3, 4, and 5 μL (for 10–50 pmole range) or 0, 10, 20, 30, 40, and 50 μL (for 100–500 pmole range) of the 0.01 mM G6P standard solution into a 96 well plate, generating 0 (blank), 0, 10, 20, 30, 40, and 50 pmole/well standards or 0 (blank), 100, 200, 300, 400, and 500 pmole/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 after deproteinization with a 10 kDa MWCO spin filter.

Tissue (10–100 mg) or cells (5×10^6) should be rapidly 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 prior to addition to the reaction.

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

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

Note: NADPH in samples will generate background for the assay. To remove the effect of NADPH background, include a blank sample for each sample by omitting the Glucose-6-Phosphate Enzyme Mix in the Reaction Mix.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 μL of the appropriate Reaction Mix is required for each reaction (well). For samples containing less than 250 pmoles of G6P, reduce the probe volume to 1 μL per well to reduce background.
2. Add 50 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the reaction for 5 minutes at 37 °C. Protect the plate from light during the incubation.

Table 1.

Reaction Mixes

Reagent	Samples and Standards	Blank Sample
G6P Assay Buffer	42 μL	44 μL
G6P Enzyme Mix	2 μL	–
G6P Substrate Mix	2 μL	2 μL
G6P Probe	4 μL	4 μL

3. Measure fluorescence intensity ($\lambda_{\text{ex}} = 535/$
 $\lambda_{\text{em}} = 587 \text{ nm}$).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Glucose-6-Phosphate 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 Glucose-6-Phosphate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, the amount of Glucose-6-Phosphate present in the sample may be determined from the standard curve.

Concentration of Glucose-6-Phosphate

$$S_a/S_v = C$$

A_y = Amount of Glucose-6-Phosphate in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells.

C = Concentration of Glucose-6-Phosphate in sample

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

Sample Calculation

Amount of Glucose-6-Phosphate (S_a) = 20 pmole

Assay volume (S_v) = 50 μL

Concentration of Fructose-6-Phosphate in sample

$$20 \text{ pmole}/50 \mu\text{L} = 0.4 \text{ nmole}/\mu\text{L}$$

$$0.4 \text{ pmole}/\mu\text{L} \times 260.14 \text{ pg/pmole} = 104.0 \text{ pg}/\mu\text{L}$$

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.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided.
	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 samples will be used 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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