

Glucose-6-Phosphate Assay Kit I

A simple, sensitive and easy colorimetric assay to detect glucose-6-phosphate concentrations

Instruction Manual

Catalog Number	PK-CA577-K657															
Description	<p>Glucose-6-phosphate (G6P) is a key sugar intermediate for glucose to get into cells, and then enter either metabolic pathways or storage. G6P can enter the glycolytic pathway, the pentose phosphate shunt or be stored as glycogen or starch. G6P is utilized by its 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.</p> <p>PromoCell's <i>Glucose-6-Phosphate Assay Kit I</i> provides a simple, sensitive and rapid tool to quantify G6P in a variety of samples. In the assay, glucose-6-phosphate is oxidized to a product which is utilized to convert a nearly colorless probe to an intensely colored product with an absorbance at 450 nm. The assay can detect G6P in the range of 1 to 30 nmoles with a detection sensitivity of ~10 μM.</p>															
Quantity	100 assays															
Applications	<ul style="list-style-type: none">• Measurement of Glucose-6-Phosphate in various tissues/cells• Analysis of glucose metabolism and cell signaling in various cells• Screening anti-diabetic drugs															
Sample Type	<ul style="list-style-type: none">• Animal tissues and cultured cells (adherent or suspension cells)															
	<table border="1"><thead><tr><th>Components</th><th>Quantity</th><th>Color Code</th></tr></thead><tbody><tr><td>G6P Assay Buffer</td><td>25 ml</td><td>WM</td></tr><tr><td>G6P Substrate Mix (lyophilized)</td><td>1 vial</td><td>Red</td></tr><tr><td>G6P Standard (10 μmol; lyophilized)</td><td>1 vial</td><td>Yellow</td></tr><tr><td>G6P Enzyme Mix (lyophilized)</td><td>1 vial</td><td>Green</td></tr></tbody></table>	Components	Quantity	Color Code	G6P Assay Buffer	25 ml	WM	G6P Substrate Mix (lyophilized)	1 vial	Red	G6P Standard (10 μmol ; lyophilized)	1 vial	Yellow	G6P Enzyme Mix (lyophilized)	1 vial	Green
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User Supplied Reagents & Equipment	<ul style="list-style-type: none">• 96-well plate with flat, clear bottom• Multi-well spectrophotometer (ELISA reader)															
Storage and Reagents Preparation	<p>Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Warm Assay Buffer to room temperature before use. Read the entire protocol before performing the assay.</p> <ul style="list-style-type: none">• G6P Enzyme Mix: Dissolve with 220 μl dH₂O. Pipette up and down to dissolve. Aliquot into portions and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.• G6P Substrate Mix: Dissolve with 220 μl of G6P Assay Buffer. Pipette up and down to dissolve. Stable for 2 months at 4°C.• G6P Standard: Dissolve in 100 μl dH₂O to generate 100 mM (100 nmol/μl) G6P Standard solution. Keep cold while in use. Store at -20°C.															
Assay Protocol	<p>1. Sample Preparation: Liquid or solution samples can be assayed directly. For tissue or cell samples: 10-100 mg tissue or 5 million cells should be rapidly homogenized with 2-3 volume of ice cold PBS or other buffer (pH 6.5-8). Centrifuge at top speed for 10 minutes to remove insoluble materials. Add 1-50 μl samples into duplicate wells of a 96-well plate and bring volume to 50 μl with Assay Buffer. For unknown samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range. Notes:</p> <p>A. Enzymes in sample may convert or consume G6P. We suggest to deproteinize samples using a perchloric acid/KOH protocol (Cat. No. PK-CA577-K808) or a 10 kDa molecular weight cut off spin filter (Cat. No. PK-CA577-1997) to remove enzymes. Samples may be homogenized in perchloric acid, then neutralize with 10N KOH to minimize G6P conversion. For tissues or cells containing low level of free G6P (5-60 μM), try to minimize sample dilutions.</p> <p>B. NADH or NADPH in samples will generate background readings. If NADH or NADPH is in your sample, you may do a background control (omit G6P Enzyme Mix from the reaction mix) to read the background, then subtracted the background from G6P readings.</p> <p>2. Standard Curve Preparations: Dilute the G6P Standard to 1 nmol/μl by adding 10 μl of the 100 nmol/μl Standard to 990 μl of dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of standards wells on a 96 well plate. Adjust volume to 50 μl/well with Assay Buffer to generate 0,</p>															

2, 4, 6, 8, 10 nmol/well of G6P Standard.

3. Develop: Mix enough reaction mix for the number of samples and standards to be performed:
For each well, prepare a total 50 μ l Reaction Mix containing:

	Reaction Mix	Background
G6P Assay Buffer	46 μ l	48 μ l
G6P Enzyme Mix	2 μ l	----
G6P Substrate Mix	2 μ l	2 μ l

Add 50 μ l of the Reaction Mix to each well containing the G6P Standard and samples. Add 50 μ l of the background mix into background control wells.

Incubate for 30 minutes at room temperature, protect from light.

4. Measurement: Read OD at 450 nm.

5. Calculation: Correct background by subtracting the value of the 0 G6P blank from all sample readings. If background control reading is significant, subtract the background reading from sample reading. Plot the standard curve. Apply the corrected sample readings to the standard curve to get G6P amount in the sample wells.

The G6P concentrations in the test samples: $C = Ay/Sv$ (nmol/ μ l; or μ mol/ml; or mM)

Where: Ay is the amount of G6P (nmol) in your sample from the standard curve.

Sv is the sample volume (μ l) added to the sample well.

Glucose-6-phosphate molecular weight: 260.14.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

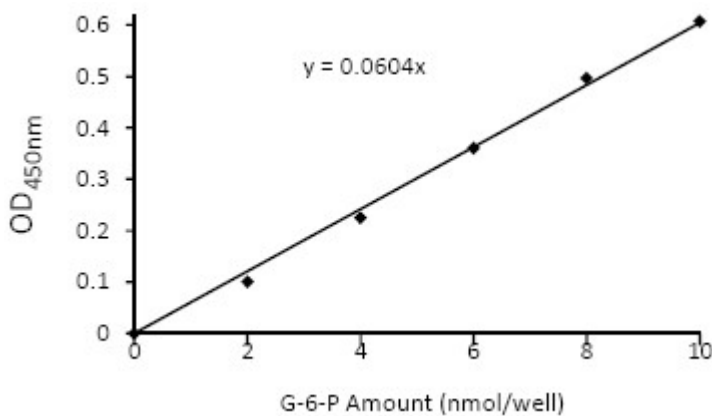


Figure: Glucose-6-phosphate standard curve generated using this kit protocol.

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