Glucose-6-Phosphate Assay Kit

(Catalog #K657-100; 100 assays; Store Kit at -20°C)

I. Introduction:

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. BioVision's glucose-6-phosphate Assay Kit is a simple, sensitive and rapid means of quantifying G6P in a variety of samples. In the assay, glucose-6-phosphate is oxidized with the generation of a product which is utilized to convert a nearly colorless probe to an intensely colored product with an absorbance at 450 nm. The Glucose-6-phosphate Assay Kit can detect G6P in the range of 1 to 30 nmoles with detection sensitivity ~10 µM of G6P.

II. Kit Contents:

Components	K657-100	Cap Code	Part Number
G6P Assay Buffer	25 ml	WM	K657-100-1
G6P Enzyme Mix	lyophilized	Green	K657-100-2
G6P Substrate Mix	lyophilized	Red	K657-100-3
G6PStandard (10 µmol)	lyophilized	Yellow	K657-100-4

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm G6P Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep enzyme mix on ice while in use.

VI. Reagent Preparation and Storage Conditions:

G6P Enzyme Mix: Dissolve with 220 µl dH_2O. 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.

V. Assay Protocol:

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 min 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:

A. Enzymes in sample may convert or consume G6P. We suggest to deproteinize samples using a perchloric acid/KOH protocol (BioVision, Cat.# K808-200) or 10 kDa molecular weight cut off spin filter (BioVision, Cat.# 1997-25) 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.

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.

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, 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 µl Reaction Mix containing:

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

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

4. Incubate for 30 min at room temperature, protect from light.

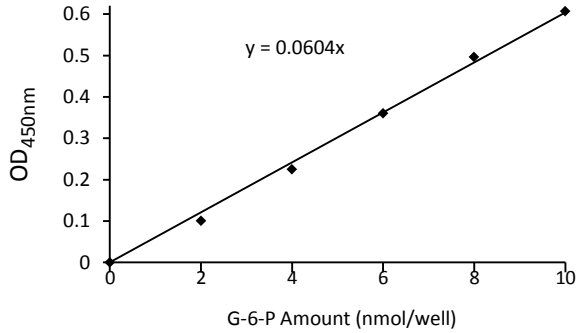
5. Measure OD at 450 nm.

6. **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/µl; or µmol/ml; or mM)

Where: Ay is the amount of G6P (nmol) in your sample from the standard curve.
Sv is the sample volume (µl) added to the sample well.
Glucose-6-phosphate molecular weight: 260.14.



Glucose-6-phoshate standard curve generated using this kit protocol

RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose and Sucrose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Lactate Assay Kits
TAC Total Antioxidant Capacity	Mono or Polysaccharide Assay Kits
Malic acid Assay Kit	Pyruvate Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		