PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit

(Catalog #K687-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Glucose-6-phosphate (G6P) is a key intermediate for glucose transport into cells which then enters 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 NAD(P)H. 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 that converts a nearly colorless probe to an intensely fluorescent product (Ex/Em = 535/587 nm). The Glucose-6-phosphate Assay Kit can detect G6P in the range of 10 to 500 pmoles which is equivalent to the range of 1-500 µM in the original sample assuming a dilution of 5X during processing. The PicoProbe™ G6P Assay Kit is much more sensitive than the G6P Colorimetric Assay Kit for low G6P samples.

II. Kit Contents:

Components	K687-100	Cap Code	Part Number
G6P Assay Buffer	25 ml	WM	K687-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K687-100-2
G6P Enzyme Mix (lyophilized)	1 vial	Green	K687-100-3
G6P Substrate Mix (lyophilized)	1 vial	Red	K687-100-4
G6P Standard (10 µmol; lyophilized)	1 vial	Yellow	K687-100-5

III. Reagent Preparation and Storage Conditions:

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.

PicoProbe™: DMSO solution. Ready to use as supplied. Warm to room temperature to thaw before using. Store at -20°C.

G6P Enzyme Mix, G6P Substrate Mix: Dissolve with 220 µl Assay Buffer. Pipette up and down to dissolve. Aliquot into portions and store at –20°C. Avoid repeated freeze/thaw cycles. Use within two months.

G6P Standard: Dissolve in 100 µl dH_2O to generate 100 mM (100 nmol/µl) G6P Standard solution. Keep on ice while in use. Store at -20°C.

IV. Assay Protocol:

1. **Sample Preparation:** Liquid 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 volumes of ice-cold Assay Buffer or other buffer (pH 6.5 - 8). Centrifuge at top speed for 2 - 3 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 consume G6P. We suggest deproteinizing samples using a perchloric acid/KOH protocol (BioVision, Cat.# K808-200) or a 10 kDa molecular weight cut off spin filter (BioVision, Cat.# 1997-25) to remove enzymes. Tissues or cells may be homogenized in 2 N perchloric acid then neutralized with 10 N KOH to minimize G6P conversion. For tissues or cells containing low levels of free G6P (< 10 µM), try to minimize sample dilution.

B. NADPH in samples will generate background readings. If NADPH is in your sample, you may do a background control (omit G6P Enzyme Mix from the reaction mix) to read the sample background, then subtract the background from G6P readings.

ates enhance the sensitivity of fluorescent asays and are highly recommended.

2. **Standard Curve Preparations:** Depending on your sample concentration, dilute the G6P Standard to 1 nmol/µl (1 mM) by adding 10 µl of the 100 nmol/µl Standard to 990 µl of dH_2O, mix well. Dilute the 1 nmol/µl standard to 10 pmol/µl by adding 10 µl to 990 µl of dH_2O. Add 0, 1, 2, 3, 4, 5 µl (for 0-50 pmol range) or 0, 10, 20, 30, 40, 50 µl (for 0-500 pmol range) into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 pmol/well or 0, 100, 200, 300, 400, 500 pmol/well of G6P Standard (see standard curve below).
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	Sample Background
G6P Assay Buffer	42 µl	44 µl
PicoProbe™ **	4 µl	4 µl
G6P Enzyme Mix	2 µl	----
G6P Substrate Mix	2 µl	2 µl

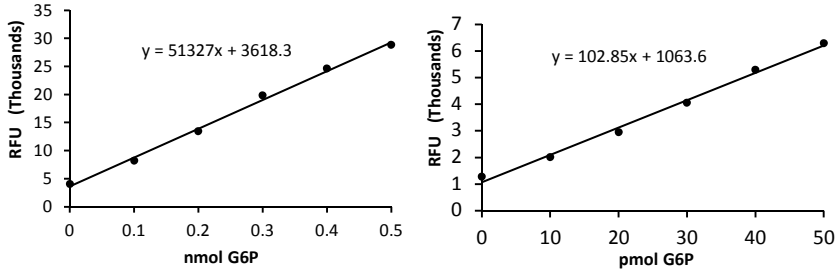
Add 50 µl of the Reaction Mix to each well containing the G6P Standard and samples. Add 50 µl of the background mix into sample background control wells.

****NOTE:** For samples containing less than 250 picomoles of G6P, reduce the probe volume to 1 µl per well to reduce reagent background and increase the reaction buffer appropriately.

4. Incubate for 5 min at 37°C, protect from light.
5. Measure fluorescence using Ex/Em = 535/587 nm with a plate reader.
6. **Calculation:** Correct reagents background by subtracting the value of the 0 G6P blank from all readings. If sample background reading is significant, subtract the sample 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 (pmol/µl; or nmol/ml; or µM)

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



G6P Standard Curves (0 – 500 and 0 – 50 pmol range) generated using this Kit Protocol.

V. RELATED PRODUCTS:

G6P Colorimetric Assay Kit
G6PDH Activity Assay Kit
Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit
Lactate Assay Kits
Glucose and Sucrose Assay Kit
Glutathione 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 or white 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.		