APExBIO
An Apoptosis and Epigenetics Company

Product Information

Glucose-6-Phosphate Fluorometric Assay Kit

I. Kit Contents:

Components	K2220-100 100 assays	Cap Color	Part Number
G6P Assay Buffer	25 ml	WM	K2220-C-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K2220-C-2
G6P Enzyme Mix (lyophilized)	1 vial	Green	K2220-C-3
G6P Substrate Mix (lyophilized)	1 vial	Red	K2220-C-4
G6P Standard (10 µmol; lyophilized)	1 vial	Yellow	K2220-C-5

II. Introduction:

Glucose-6-phosphate (G6P) is an important intermediate for glucose to get into cells. G6P is involved in pentose phosphate pathway, glycolytic pathway or be stored as glycogen or starch. G6P is utilized by glucose 6-phosphate dehydrogenase to produce NAD(P)H, which is very important in red blood cells. The deficiency of G6PDH will lead to hemolytic anemia.

The Glucose-6-Phosphate Fluorometric Assay Kit provides a highly sensitive, simple, fast and convenient way for detection of G6P levels in various biological samples based on fluorometric method. In the assay, G6P is oxidized to generate a product which converts a nearly colorless probe to an intensely fluorescent product (Ex/Em 535/587 nm). The kit can detect 10 to 500 pmoles G6P which is equivalent to the range of 1 - 500 µM in the original sample assuming a dilution of 5 × during processing. The kit is more sensitive than the G6P Colorimetric Assay Kit for low G6P samples.

III. Storage and Handling:

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 or a 10 kDa molecular weight cut off spin filter 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.

C. White plates 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.

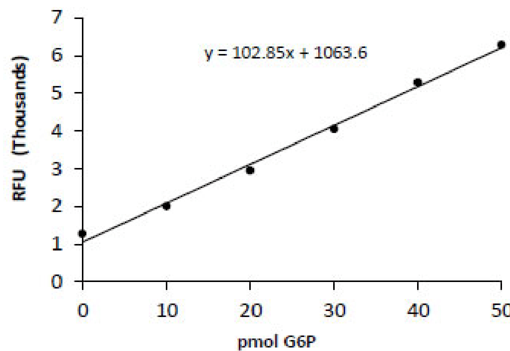
6. Calculation: Correct reagents background by subtracting the value of the 0 G6P blank from all readings. If sample background reading is significant, subtract the sample background reading from sample reading. Plot the standard curve. Apply the corrected sample readings to the standard curve to get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

$$C = Ay/Sv \text{ (pmol/µl; or nmol/ml; or µM)}$$

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.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely

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

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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