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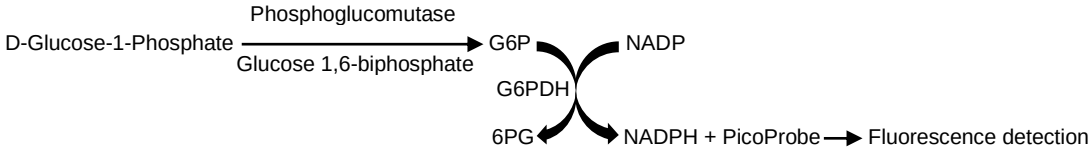
PicoProbe™ Phosphoglucomutase Assay Kit (Fluorometric)

5/15

(Catalog # K770-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphoglucomutase (PGM) enzyme plays a key role in carbohydrate metabolism and widely exists in all organisms. PGM interconverts Glucose-1-Phosphate (G1P) and Glucose-6-Phosphate (G6P) depending on the body requirement. When glycogen breaks down, G1P is generated and phosphoglucomutase converts G1P to G6P, which can go either to glycolytic pathway to generate ATP, or to pentose phosphate pathway to generate ribose and NADPH. On the other hand, when cells have extra energy, PGM converts G6P to G1P, which generates glycogen. In humans, phosphoglucomutase has 2 isoforms (PGM I and PGM II). PGM deficiency leads to glucose storage disease. Detection of abnormal phosphoglucomutase activity is crucial for diagnosis, prediction and treatment of the disease. In BioVision's phosphoglucomutase assay, PGM converts glucose-1-phosphate to glucose-6-phosphate; the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADPH, which reduces PicoProbe™ to generate an intense fluorescence product (Ex/Em = 535/587 nm). This assay kit is simple, highly sensitive, and rapid and can detect as low as 20 µU of PGM activity.



II. Applications:

- Measurement of phosphoglucomutase activity in various tissues/cells.
- Analysis of glucose metabolism and cell signaling in various cell types.
- Screening of anti-diabetic drugs.

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney etc.
- Cell culture: adherent or suspension cells.
- Plasma.

IV. Kit Contents:

Components	K770-100	Cap Code	Part Number
PGM Assay Buffer	25 ml	WM	K770-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K770-100-2
PGM Substrate	1 vial	Blue/White Dot	K770-100-3
PGM Enzyme Mix	1 vial	Green	K770-100-4
PGM Developer	1 vial	Red	K770-100-5
NADPH Standard (200 nmol)	1 vial	Yellow	K770-100-6
PGM Positive Control	1 vial	Purple	K770-100-7

V. User Supplied Reagents and Equipment:

- 96-well flat bottom plate. White plate is preferred for this assay.
- Multi-well spectrophotometer (fluorescence reader)

VI. Reagent Preparation and Storage Conditions:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **PGM Assay Buffer:** Warm to room temperature before use. Store at -20°C or 4°C.
- **PicoProbe™**: Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **PGM Substrate:** Reconstitute with 220 µl Assay Buffer to generate 0.2 M solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **PGM Enzyme Mix and PGM Developer:** Reconstitute each with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
- **NADPH Standard:** Reconstitute with 160 µl dH_2O to generate 1.25 mM NADPH Standard solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **PGM Positive Control:** Reconstitute with 100 µl Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

VII. Phosphoglucomutase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (50-100 mg) or cells (1-5 x 10^6) with 200 µl ice cold Assay Buffer and keep the homogenate on ice for 5 min. Centrifuge at 12,000 rpm, 4°C for 5 min. Collect the supernatant. Add 2-10 µl sample (~0.2-1 µg protein) per well. Dilute 1 µl of the Positive Control solution into 999 µl Assay Buffer. Use 1-10 µl of the diluted Positive Control into desired well(s). Adjust the volume of Positive Control and Sample wells to 50 µl/well with Assay Buffer.

   Notes:

a. For unknown samples, we suggest doing pilot experiment & testing several dilutions to ensure the readings are within the Standard Curve range.

b. If necessary, protease inhibitor can be added during sample preparation (Cat. # K271 or similar).

c. For samples having high NADPH background, prepare parallel sample well(s) as the sample background control(s).

2. **NADPH Standard Curve:** Add 2 µl of the 1.25 mM NADPH Standard to 198 µl of Assay buffer to make 12.5 µM solution. Add 0, 2, 4, 6, 8, and 10 µl of 12.5 µM NADPH Standard into a series of wells in a 96-well plate to generate 0, 25, 50, 75, 100 and 125 pmol/well of NADPH Standard. Adjust the volume to 50 µl/well with Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl mix containing:

	Reaction Mix	Background Control Mix
PGM Assay Buffer	43 µl	45 µl
PicoProbe™	1 µl	1 µl
PGM Enzyme Mix	2 µl	2 µl
PGM Developer	2 µl	2 µl
PGM Substrate	2 µl	----

Add 50 µl of the reaction mix to each well containing the Standard, Positive Control, and samples. Mix well.

Note: Add 50 µl of background control mix to sample background control well.

4. **Measurement:** Incubate for 10-60 min. at room temperature and measure fluorescence (Ex/Em = 535/587 nm).

Notes:

a. Incubation time depends on the phosphoglucomutase activity in the samples. We recommend measuring RFU in a kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the phosphoglucomutase activity of the samples. The NADPH Standard Curve can be read in end point mode (i.e., at the end of incubation time).

b. To express specific PGM activity as mU/mg protein, measure protein concentration of samples with appropriate kit (Cat. # K813 or similar).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading. Calculate the phosphoglucomutase activity of the sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the NADPH Standard Curve to get B pmol of NADPH generated during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample Phosphoglucomutase Activity} = \frac{B}{\Delta T \times M} \textbf{ x Dilution Factor} = \left(\frac{pmol}{min.\mu g}\right) \textbf{ or } \left(\frac{mU}{mg}\right)$$

Where: **B** is amount of NADPH in the sample well from Standard Curve (pmol)
ΔT is the incubation time (min.)
M is the protein amount added into the reaction well (µg)

Unit Definition: One unit of phosphoglucomutase is the amount of enzyme that generates 1.0 µmol of NADPH per min. at pH 8 at room temperature.

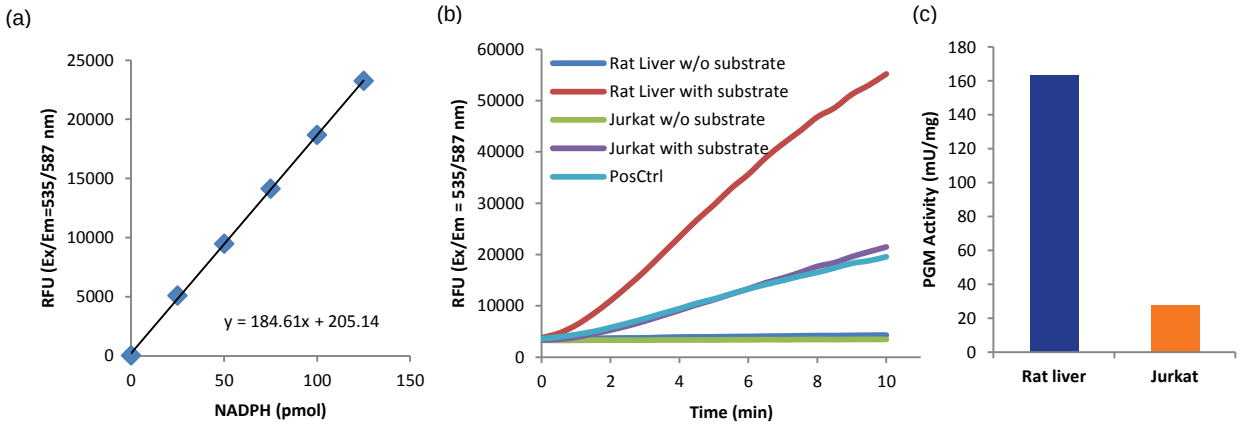


Figure: NADPH standard curve (a). Phosphoglucomutase activity in Jurkat cell lysate (0.4 µg), rat liver homogenate (0.2 µg), and Positive Control (b). Phosphoglucomutase specific activity in Jurkat cell lysate and rat liver homogenate (c). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Glucose Assay kit (K606)
Glucose Dehydrogenase Activity Assay Kit (K786)
Glucose-1-Phosphate Colorimetric Assay Kit (K697)
Glucose Uptake Colorimetric Assay Kit (K676)
Glycogen Assay Kit (K646)
Maltose and Glucose Assay Kit (K618)
Glucose and Sucrose Assay Kit (K616)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Glucose Uptake Fluorometric Assay Kit (K666)
Hexokinase Colorimetric Assay Kit (K789)
GluTracker™ Glucose Uptake Assay Kit (Cell-Based) (K681)

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