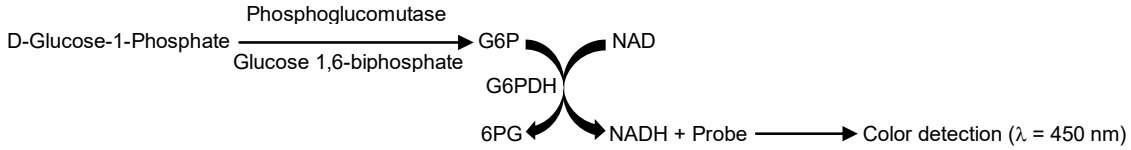
Phosphoglucomutase Colorimetric Assay Kit (#BN00987)

(Catalog # BN00987; 100 assays; Store at -20°C)

I. Introduction:

Phosphoglucomutase (PGM) 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 have 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 Assay Genie'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 NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. The Phosphoglucomutase Assay Kit is simple, sensitive and rapid and can detect PGM activity even less than 1 mU/reaction.



II. Application:

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

III. Sample Type:

- Animal tissues: Muscle, Liver, heart, kidney etc.
- Cell culture: Adherent or suspension cells.
- Plasma

IV. Kit Contents:

Components	BN00987	Cap Code	Part Number
PGM Assay Buffer	27 ml	WM	BN00987-1
PGM Substrate (lyophilized)	1 vial	Blue	BN00987-2
PGM Enzyme Mix (Lyophilized)	1 vial	Green	BN00987-3
PGM Developer (Lyophilized)	1 vial	Red	BN00987-4
NADH Standard (Lyophilized)	1 vial	Yellow	BN00987-5
PGM Positive Control (Lyophilized)	1 vial	Purple	BN00987-6

V. User Supplied Reagents and Equipment:

- 96-well plate with flat clear bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **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:** Reconstitute 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.
- **PGM Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH 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.

VIII. Phosphoglucomutase Assay Protocol:

1. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells of 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50 µl/well with Assay Buffer.
2. **Sample Preparation:** Rapidly homogenize tissue (50 mg) or cells (5 x 10^6) with 200 µl ice cold Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (~50-100 µg) per well, adjust final volume to 50 µl with Assay Buffer. For samples having high NADH background, prepare a parallel sample well as the background control to avoid interference from the NADH in the sample.

Notes: a) Reducing small molecules in some tissue samples such as liver may interfere with PGM assay. We recommend removing the small molecules by ammonium sulfate precipitation method. Ammonium sulfate precipitation: Aliquot 50-100 µl (~300-500 µg) of tissue sample to a clean centrifuge tube & add saturated ammonium sulfate to final concentration of 3.2 M. Incubate on ice for 20 min. Centrifuge at 14,000 rpm for 5 mins. Discard the supernatant and resuspend the pellet to the original 50-100 µl volume. b) For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

3. **Positive Control:** Dilute Positive Control solution 1:99 in Assay Buffer. Use 1-10 µl of diluted Positive Control into the desired well(s) & adjust final volume to 50 µl with Assay Buffer.
4. **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	44 µl	46 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 test samples and 50 µl of background control mix to each well containing the background control sample. Mix well.
Note: Background control mix is recommended for samples having high NADH background.

5. **Measurement:** Incubate for 20-60 min at room temperature and measure OD_{450nm}.
Note: Incubation time depends on the phosphoglucomutase activity in the samples. We recommend measuring the OD in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the phosphoglucomutase activity of the samples. The NADH standard curve can read in Endpoint mode (i.e., at the end of incubation time).
6. **Calculation:** Subtract the 0 standard reading from all readings. Plot the NADH standard curve. Correct sample background by subtracting the value derived from the background control from sample readings. Calculate the phosphoglucomutase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

Sample Phosphoglucomutase activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml = mU/ml

Where: **B** is the NADH amount from the standard curve (nmol).
ΔT is the reaction time (min).
V is the sample volume added into the reaction well (ml).

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

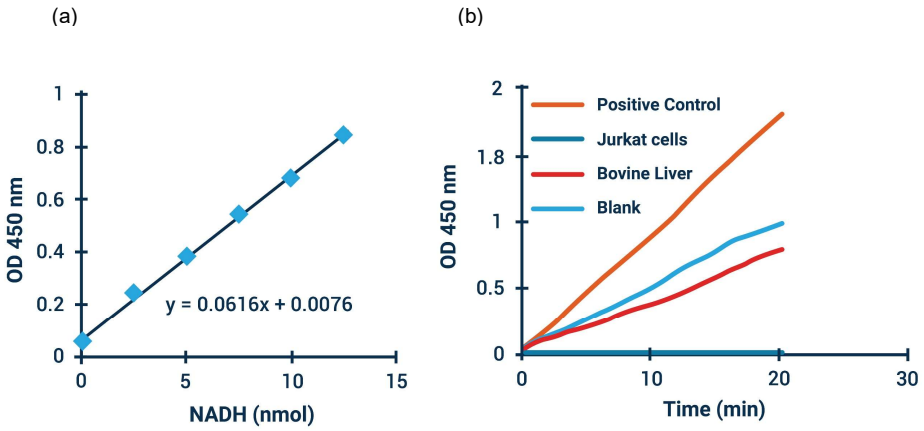


Figure: NADH standard curve (a). Phosphoglucomutase activity in pure PGM Positive Control, jurkat cell lysate (induced with 2 µM camptothecin) and bovine liver lysate respectively (b). Assays were performed following kit protocol.

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