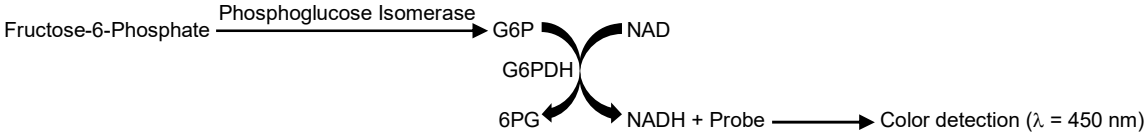
Phosphoglucose Isomerase Activity Colorimetric Assay Kit

(Catalog # BN00988; 100 assays; Store at -20°C)

I. Introduction:

Phosphoglucose isomerase (PGI, EC 5.3.1.9) is an important housekeeping enzyme. PGI catalyzes the interconversion of glucose-6-phosphate to fructose-6-phosphate. PGI performs multiple functions & intracellularly plays key role in both glycolysis and gluconeogenesis. Extracellularly, PGI [also called Autocrine Motility Factor (AMF)] functions as a cytokine, which stimulates cell motility and is associated with tumor development and metastasis. In humans, PGI deficiency causes hemolytic anemia, whereas increased PGI activity is observed in many cancers such as gastrointestinal, kidney and breast cancer. Early detection of abnormal phosphoglucose isomerase activity is crucial for diagnosis, prediction and therapeutic strategy. In Assay Genie's Phosphoglucose Isomerase Activity Assay Kit, PGI converts fructose-6-phosphate to glucose-6-phosphate; the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form a product, which reacts with a colorless probe to give strong absorbance at 450 nm. The PGI assay is simple, sensitive and rapid and can detect phosphoglucose isomerase activity less than 0.1 mU/reaction.



II. Application:

- Measurement of phosphoglucose isomerase 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: Liver, brain, heart, kidney etc.
- Cell culture: Adherent or suspension cells
- Bacteria, yeast, fish etc.

IV. Kit Contents:

Components	BN00988	Cap Code	Part Number
PGI Assay Buffer	25 ml	WM	BN00988-1
PGI Substrate (Lyophilized)	1 vial	Blue	BN00988-2
PGI Enzyme Mix (Lyophilized)	1 vial	Green	BN00988-3
PGI Developer (Lyophilized)	1 vial	Red	BN00988-4
NADH Standard (Lyophilized)	1 vial	Yellow	BN00988-5
PGI Positive Control (Lyophilized)	1 vial	Purple	BN00988-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Ammonium sulfate solution (saturated, 4.1 M)

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **PGI Substrate:** Reconstitute with 220 µl Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.
- **PGI 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.
- **PGI 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 40 µl Assay buffer to generate 12.5 mM (12.5 nmol/µl) NADH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.
- **PGI Positive Control:** Reconstitute with 20 µl Assay Buffer and mix thoroughly. Aliquot and store at –20°C. Keep on ice while in use.

VIII. Phosphoglucose Isomerase Assay Protocol:

1. **NADH Standard Curve:** Dilute NADH Standard 1:10 by adding 5 µl of 12.5 mM NADH Standard to 45 µl of Assay Buffer to generate 1.25 mM NADH Standard. Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in 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 (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 subtract interference from the NADH in the sample.
   Notes: a) Reducing small molecules in some samples may interfere with PGI assay. We recommend removing the small molecules by ammonium sulfate precipitation method. Ammonium sulfate precipitation: Aliquot 10-100 µl (~300-500 µg) of sample to a clean

centrifuge tube & add saturated ammonium sulfate (~4.1 M) to final concentration of 3.2 M. Incubate on ice for 20 min. Centrifuge at 14,000 rpm for 5 mins. Suspend the pellet to the original 10-100 μl volume. b) For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

3. **PGI Positive Control:** Make fresh dilution of Positive Control by adding 2 µl Positive control to 998 µl dH_2O. 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*
PGI Assay Buffer	44 µl	46 µl
PGI Enzyme Mix	2 µl	2 µl
PGI Developer	2 µl	2 µl
PGI 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 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 phosphoglucose isomerase activity in the samples. We recommend measuring OD in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the PGI activity of the samples. The NADH Standard Curve can read in endpoint mode (i.e., at the end of incubation time).
6. **Calculation:** Subtract 0 Standard reading from all standard readings. Plot the NADH Standard Curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Calculate the phosphoglucose isomerase 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 Phosphoglucose Isomerase activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml = mU/ml

Where: **B** is the NADH amount from Standard Curve (nmol).
V is the sample volume added into the reaction well (ml).
Δ**T** is the reaction time (min).

Unit Definition: One unit of phosphoglucose isomerase is the amount of enzyme that generates 1.0 µmol of NADH/min at pH 8.0 at 25°C.

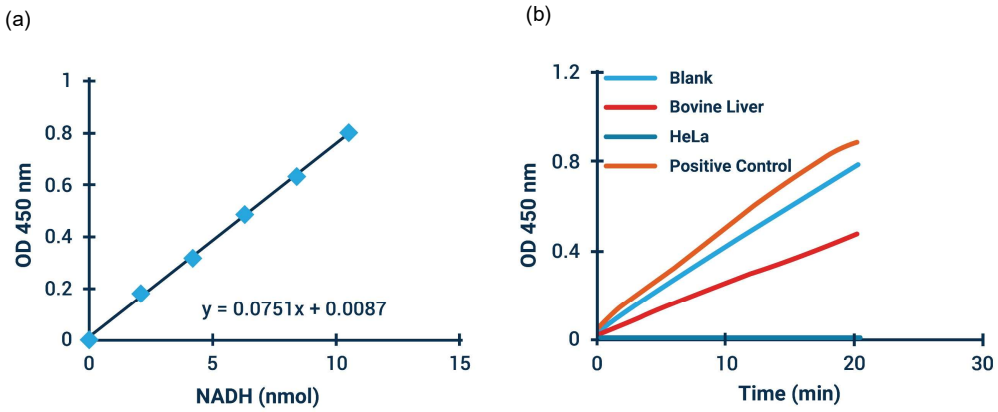


Figure: NADH Standard Curve (a). Phosphoglucose Isomerase activity in bovine liver, HeLa cell lysate & Positive Control (b). Assays were performed following kit protocol.

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