ab155897

Glucose-6-phosphate Isomerase Activity Assay Kit (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of Glucose-6-phosphate Isomerase activity in various biological samples and in screening of anti-diabetic drugs.

View kit datasheet: www.abcam.com/ab155897

(use www.abcam.cn/ab155897 for China, or www.abcam.co.jp/ab155897 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version: 3 Last Updated: 12 October 2023

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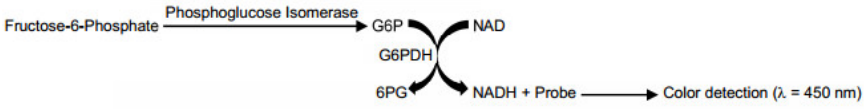
1. Overview

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 Abcam's glucose-6-phosphate 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.



2. Protocol Summary

Reagent Preparation

Standard Curve Preparation

Sample Preparation

Positive Control

Reaction Mix

Measurement and Calculation

3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer V/PGI Assay Buffer	27 mL
Phosphoglucose Isomerase Substrate/PGI Substrate (Lyophilized)	1 vial
Development Enzyme Mix IX/PGI Enzyme Mix (Lyophilized)	1 vial
Developer Solution III/PGI Developer (Lyophilized)	1 vial
NADH Standard I/NADH Standard (Lyophilized)	1 vial
Phosphoglucose Isomerase Positive Control/PGI Positive Control (Lyophilized)	1 vial

* Store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

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

4. Assay Protocol

A. Reagent Preparation

1. Phosphoglucose Isomerase Substrate/PGI Substrate:
Reconstitute with 220 µl Assay Buffer V/Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.

2. Development Enzyme Mix IX/PGI Enzyme Mix:
Reconstitute with 220 µl Assay Buffer V/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.

3. Developer Solution III/PGI Developer:
Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

4. NADH Standard I/NADH Standard:
Reconstitute with 40 µl Assay Buffer V/Assay buffer to generate 12.5 mM (12.5 nmol/µl) NADH Standard I/NADH Standard

solution. Store at –20°C. Use within two months. Keep on ice while in use.

5. **Phosphoglucose Isomerase Positive Control/PGI Positive Control:**
   Reconstitute with 20 µl Assay Buffer V/Assay Buffer and mix thoroughly. Aliquot and store at –20°C. Keep on ice while in use.

B. Glucose-6-phosphate Isomerase Assay Protocol

1. **NADH Standard I/NADH Standard Curve:**
   Dilute NADH Standard I/NADH Standard 1:10 by adding 5 µl of 12.5 mM NADH Standard I/NADH Standard to 45 µl of Assay Buffer V/Assay Buffer to generate 1.25 mM NADH Standard I/NADH Standard. Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard I/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 I/NADH Standard. Adjust volume to 50 µl/well with Assay Buffer V/Assay Buffer.

2. **Sample Preparation:**
   Rapidly homogenize tissue (50 mg) or cells (5 x 10^6) with 200 µl ice cold Assay Buffer V/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 V/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. **Positive Control:**

   Make fresh dilution of Positive Control by adding 2 µl Phosphoglucose Isomerase Positive Control/Positive control to 998 µl dH2O. Use 1-10 µl of diluted Phosphoglucose Isomerase Positive Control/Positive Control into the desired well(s) and adjust final volume to 50 µl with Assay Buffer V/Assay Buffer.

4. Reaction Mix:

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

	Reaction Mix	Background Control Mix*
Assay Buffer V/PGI Assay Buffer	44 µl	46 µl
Development Enzyme Mix IX/PGI Enzyme Mix	2 µl	2 µl
Developer Solution III/PGI Developer	2 µl	2 µl
Phosphoglucose Isomerase Substrate/PGI	2 µl	---

Substrate

Add 50 µl of the reaction mix to each well containing the standard, Phosphoglucose Isomerase Positive Control/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 I/NADH Standard Curve can read in endpoint mode (i.e., at the end of incubation time).

5. Data Analysis

Calculation: Subtract the 0 standard reading from all readings. Plot the NADH Standard I/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 I/NADH standard curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample Phosphoglucose Isomerase Activity} = \frac{B}{(\Delta T \times V)} \times \text{Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

B is the amount of NADH from 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 phosphoglucose isomerase is the amount of enzyme that generates 1.0 µmol of NADH/min at pH 8.0 at 25°C.

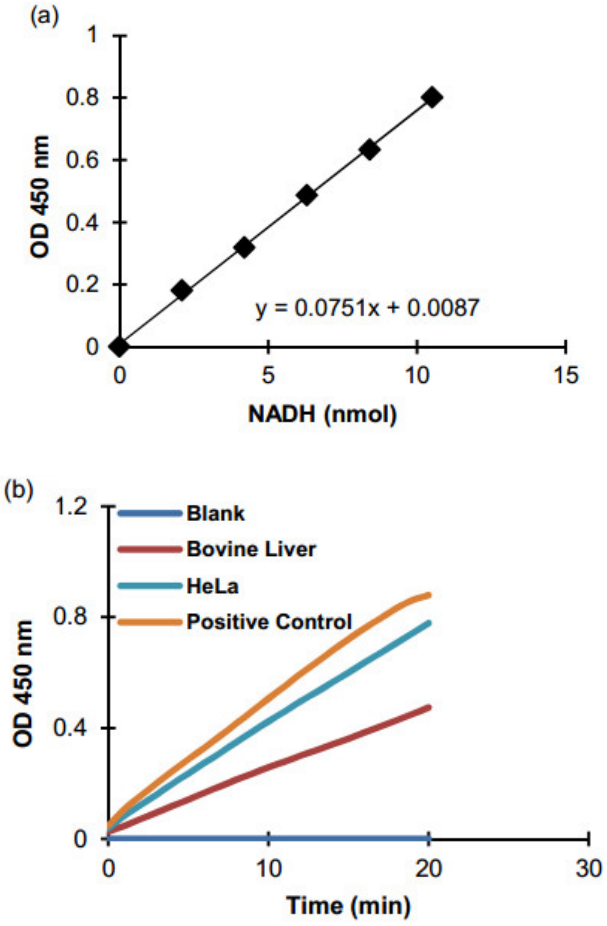


Figure 1: (a) NADH Standard I/NADH standard curve, (b) Phosphoglucose Isomerase activity in bovine liver, HeLa cell lysate and Phosphoglucose Isomerase Positive Control/positive control. Assays were performed following kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet

Problem	Reason	Solution
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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