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Product Information

Phosphoglucose Isomerase Colorimetric Assay Kit

Catalog Number **MAK103**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Phosphoglucose Isomerase (PGI) is an enzyme crucial for the interconversion of D-glucose 6-phosphate and D-fructose 6-phosphate in the second step of glycolysis and is involved in glucogenesis. PGI is also referred to as Autocrine Motility Factor (AMF) and is secreted by cancer cells in order to stimulate metastasis. Deficiencies in PGI activity can lead to hemolytic anemia.

The Phosphoglucose Isomerase Colorimetric Assay kit provides a simple and direct procedure for measuring PGI activity in a variety of samples. PGI activity is determined by a coupled enzyme assay in which fructose-6-phosphate is converted by PGI to glucose-6-phosphate. Glucose-6-phosphate is subsequently oxidized to form a product, which reacts with a probe generating a colorimetric (450 nm) product proportional to the PGI activity present. One unit of PGI is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8.0 at room temperature.

Components

The kit is sufficient for 100 assays in 96 well plates.

PGI Assay Buffer Catalog Number MAK103A	27 mL
PGI Substrate Catalog Number MAK103B	1 vl
PGI Enzyme Mix Catalog Number MAK103C	1 vl
PGI Developer Catalog Number MAK103D	1 vl
NADH Standard Catalog Number MAK103E	1 vl
PGI Positive Control Catalog Number MAK103F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Ammonium sulfate (Catalog Number A4418 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

PGI Assay Buffer – Allow buffer to come to room temperature before use.

PGI Substrate – Reconstitute with 220 μL of PGI Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

PGI Enzyme Mix – Reconstitute with 220 μL of PGI Assay buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

PGI Developer – Reconstitute with 220 μL of water. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

PGI Positive Control – Reconstitute with 20 μL of PGI Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute with 40 µL of PGI Assay Buffer to generate a 12.5 mM NADH stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Dilute 5 µl of the 12.5 mM NADH Standard with 45 µL of PGI Assay Buffer to prepare a 1.25 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM standard solution into a 96 well plate, generating 0 (blank), 2.5, 5.0, 7.5, 10, and 12.5 nmole/well standards. Add PGI Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (50 mg) or cells (5×10^6) can be homogenized in 200 µL of ice-cold PGI Assay Buffer. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material.
Note: Reducing small molecules may interfere with the assay. It is recommended to remove the small molecules by ammonium sulfate precipitation method (ammonium sulfate not provided).

Ammonium Sulfate Precipitation
Prepare saturated ammonium sulfate solution (4.1 M at 25 °C). Aliquot 10–100 µl (300–500 µg) of sample to a clean tube and add saturated ammonium sulfate solution to a final $(NH_4)_2SO_4$ concentration of 3.2 M. Mix well by pipetting and incubate on ice for 20 minutes. Centrifuge the samples at 16,000 × *g* for 5 minutes. Resuspend the pellet to original volume with PGI Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Add 1–50 µL of sample to duplicate wells. Bring samples to a final volume of 50 µL with PGI Assay Buffer.

Note: NADH in the samples can generate a background signal. To remove the effect of NADH background, a sample blank may be set up for each sample by omitting the PGI Substrate from the reaction mix.

Positive Control
For the positive control, dilute 2 µl of PGI positive control with 998 µl of water. Add 1–10 µL of the diluted PGI positive control solution to wells and adjust to 50 µL with the PGI Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
PGI Assay Buffer	44 µL	46 µL
PGI Enzyme Mix	2 µL	2 µL
PGI Developer	2 µL	2 µL
PGI Substrate	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation

3. Incubate the plate at room temperature. After 5 minutes ($T_{initial}$), measure the absorbance at 450 nm $(A_{450})_{initial}$.
   Note: It is essential $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at room temperature taking measurements (A_{450}) every 2–3 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement $[(A_{450})_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH standard from the final measurement [$(A_{450})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the final blank sample value from the final sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of NADH present in the samples may be determined from the standard curve.

Using the corrected measurements, calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of NADH generated between $T_{initial}$ and T_{final} (B).

The PGI activity of a sample may be determined by the following equation:

$$\text{PGI Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

PGI activity is reported as nmole/min/mL = milliunit/mL
One unit of PGI is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8.0 at room temperature.

Example:
NADH amount (B) = 5.84 nmole
First reading ($T_{initial}$) = 3 minute
Second reading (T_{final}) = 32 minutes
Sample volume (V) = 0.01 mL
Sample dilution is 1

PGI activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.01} = 20.14 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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