Phosphoglucose Isomerase Colorimetric Assay Kit

Cat. No. Kit-0681 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	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 (510 nm) product proportional to the PGI activity present. One unit of PGI is the amount of enzyme that will generate 1.0 mmole of NADH per minute at pH 8.0 at room temperature.
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.
Components	The kit is sufficient for 500 assays in 96 well plates. PGI kit-R1(G6PDH/DI): 85 mL PGI kit-R2(NAD/INT): 30 mL Frucose-6-Phosphate(F6P): 23 vials (0.2 mL H20 reconstitution per vial and mix with 3.8 mL PGI kit-R1 before using) NADH Standard: 5 vials PGI Positive Control: 5 vials (0.2 mL H2O reconstitution before using)
Reagents and Equipment Required but Not Provided	1. 96 well flat-bottom plate: It is recommended to use clear plates for colorimetric assays. 2. Spectrophotometric multiwell plate reader.
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

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	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 kit-R1/2: Allow to come to room temperature before use. Frucose-6-Phosphate: reconstitution per vial F6P with 3.8 mL PGI kit-R1 before using. Mix well by pipetting, then aliquot and store, protected from light, at 2-8 centgirade. Use within 2 months of reconstitution and keep cold while in use. NADH Standard: Reconstitute with 500 mL of 50mM Tris buffer (pH 9.0) to generate a 12.5 mM NADH stock solution. Mix well by pipetting, then aliquot and store at –20 centigrade. Use within 2 months of reconstitution.
Procedure	All samples and standards should be run in duplicate. NADH Standards for Colorimetric Detection: Dilute 5 ml of the 12.5 mM NADH Standard with 45 mL of PGI Assay Buffer to prepare a 1.25 mM standard solution. Add 0, 2, 4, 6, 8, and 10 mL 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 mL. Sample Preparation: Tissue (50 mg) or cells (5×10^6) can be homogenized in 200 mL 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). 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 m L of sample to duplicate wells. Bring samples to a final volume of 50 mL 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 ml of PGI positive control with 998 ml of water. Add 1–10 mL of the diluted PGI positive control solution to wells and adjust to 50 m L with the PGI Assay Buffer.

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	Assay Reaction: 1. Set up the Reaction Mixes according to the scheme in Table 1. 50 mL of the appropriate Reaction Mix is required for each reaction (well). 2. Add 36 mL of the PGI Assay Reagent 1 and 2 mL sample or cal or H2O toto 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 (Tinitial), measure the absorbance at 510 nm (A510)initial. Note: It is essential (A510)initial is in the linear range of the standard curve. 4. Continue to incubate the plate at room temperature taking measurements (A510) 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 [(A510)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 Tfinal. Note: It is essential the final measurement falls within the linear range of the standard curve.
Result	Calculations Correct for the background by subtracting the final measurement [(A510) final] obtained for the 0 (blank) NADH standard from the final measurement [(A510) 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 Tinitial to Tfinal for the samples. ΔA510 = (A510)final – (A)initial Compare the DA450 of each sample to the standard curve to determine the amount of NADH generated between Tinitial and Tfinal (B). The PGI activity of a sample may be determined by the following equation: PGI Activity = (B × Sample Dilution Factor)/[(Reaction Time) × V] B = Amount (nmole) of NADH

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	generated between Tinitial and Tfinal. Reaction Time = Tfinal – Tinitial (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 mmole of NADH per minute at pH 8.0 at room temperature. Example: NADH amount (B) = 5.84 nmole First reading (Tinitial) = 3 minute Second reading (Tfinal) = 10 minutes Sample volume (V) = 0.01 mL Sample dilution is 1. PGI activity is: (5.84×1)/[(32–3)×0.01] = 83.43 milliunits/mL
Storage	Storage at –20 centigrade, protected from light is recommended.
Shipping	On wet ice
	PGI Fructose-6-phosphate ----------→ Glucose-6-phosphate G6PDH Glucose-6-phosphate + NAD^+ ---------→ Gluconolactone-6-phosphate + NADH + H^+ Diaphorase NADH + H^+ + INT(oxidized) ---------→ NAD + H_2O + INT (reduced) color cpd. 510nm
Table 1	**Table 1.** Reaction Mixes

Reagent	Samples and Standards	Sample Blank
PGI Assay Reagent 1	36 µL	36 µL
sample or cal	2 µL	
H2O		2 µL
PGI Assay Reagent 2	12 µL	12µL

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