TECHNICAL MANUAL

Phosphoglucose Isomerase (PGI) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0518
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add measuring working solution
- Add chromogenic agent
- Incubate and measure OD values

2. Intended use

This kit can be used to measure phosphoglucose isomerase (PGI) activity in serum, plasma, animal tissue and cell samples.

3. Detection principle

Phosphoglucose Isomerase (PGI) is an important enzyme that catalyzes the interconversion between glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P), and is involved in glycolysis and the pentose phosphate pathway. These processes help cells break down glucose for energy and generate key substances in other metabolic pathways.

The detection principle of this kit is as follows: PGI catalyzes the substrate to produce a product, which reacts with a chromogenic agent to form a chromogenic substance that has maximum absorption at a wavelength of 450 nm. The PGI enzyme activity is calculated by measuring the OD value at 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	55 mL x 1 vial	55 mL x 2 vials	-20 °C, 12 months, shading light
Reagent 2	Buffer Solution	55 mL x 1 vial	55 mL x 2 vials	-20 °C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Substrate	0.5 mL x 1 vial	1 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 4	Oxidant Reagent	0.5 mL x 1 vial	1 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 5	Enzyme Reagent	0.3 mL x 1 vial	0.6 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL x 1 vial	1.5 mL x 2 vials	-20 °C, 12 months, shading light
Reagent 7	5 mmol/L Standard Solution	0.5 mL x 1 vial	1 mL x 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (445-460 nm, optimum wavelength: 450 nm), Incubator

6. Reagent preparation

1. Equilibrate all the reagents to 25 °C before use.
2. **Preparation of measuring working solution:** Before testing, prepare sufficient measuring working solution according to the test wells. For example, prepare 510 µL of measuring working solution (mix well 470 µL of buffer solution, 15 µL of substrate, 15 µL of oxidant reagent and 10 µL of enzyme reagent). The measuring working solution should be prepared on site, protected from light, and used within the same day.
3. **Preparation of 0.5 mmol/L standard solution:** Before testing, prepare sufficient 0.5 mmol/L standard solution. For example, prepare 1000 µL of 0.5 mmol/L standard solution (mix well 900 µL of double distilled water and 100 µL of 5 mmol/L standard solution). The 0.5 mmol/L standard solution should be prepared on site, protected from light, and used within the same day.

4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.15, 0.20, 0.30, 0.35, 0.40, 0.50 mmol/L.

7. Sample preparation

Sample preparation:

Serum or plasma samples: detect directly.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Homogenize 20 mg tissue in 180 µL extraction solution with a dounce homogenizer at 4 °C.

3. Centrifuge at 10000 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

4. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 x 10^6 cells).

2. Homogenize 1 x 10^6 cells in 200 µL extraction solution with an ultrasonic cell disruptor at 4 °C.

3. Centrifuge at 10000 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

4. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only). The diluent is extraction solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

Sample type - Dilution factor:

Human serum: 10-40

Rat serum: 10-40

Mouse serum: 10-40

10% Mouse liver tissue homogenate: 100-200

10% Mouse kidney tissue homogenate: 100-200

10% Mouse muscle tissue homogenate: 100-200

1 x 10^6 HL-60 cells: 10-20

1 x 10^6 293T cells: 10-20

1 x 10^6 Jurkat cells: 10-20

8. Operating steps

1. Standard well: Add 20 µL of standard solution with different concentrations to standard wells. Sample well: Add 20 µL of sample to sample wells.
2. Add 100 µL of measuring working solution into each well.
3. Add 20 µL of chromogenic agent into each well.
4. Mix fully with microplate reader and measure the OD values of each well at 450 nm, as A1. Incubate at 37 °C for 10 min and measure the OD value of each well at 450 nm, as A2. (The standard curve is fitted to the standard well A2 value.)

9. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean A_2 value of the blank (Standard #1) from all standard readings. This is the absolute A_2 value.

3. Plot the standard curve by using absolute A_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that hydrolyzes the substrate to produce 1 µmol of product at 37 °C is defined as 1 unit.

PGI activity (U/L) = (deltaA$_{450}$ - b) ÷ a ÷ T × f × 1000

2. Tissue samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that hydrolyzes the substrate to produce 1 µmol of product at 37 °C is defined as 1 unit.

PGI activity (U/gprot) = (deltaA$_{450}$ - b) ÷ a ÷ T × f ÷ C_{pr} × 1000

[Note]

deltaA$_{450}$: the absolute OD value of the sample wells, A_2 - A_1.

T: Reaction time, 10 min.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

1000: 1 mmol/L = 1000 μmol/L

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	10.0	20.0	40.0
%CV	2.0	2.7	2.3

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	10.0	20.0	40.0
%CV	6.5	8.8	9.9

Recovery

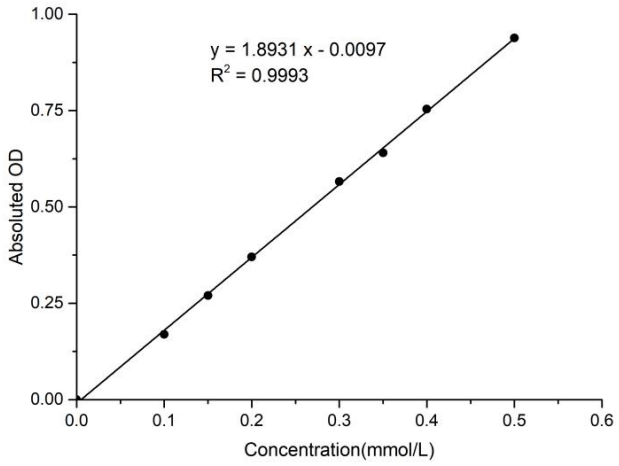
	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	10.0	20.0	40.0
Observed Conc. (U/L)	10.2	20.6	44.4
Recovery rate (%)	102	103	111

Sensitivity

The analytical sensitivity of the assay is 0.78 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



11. Appendix II Example Analysis

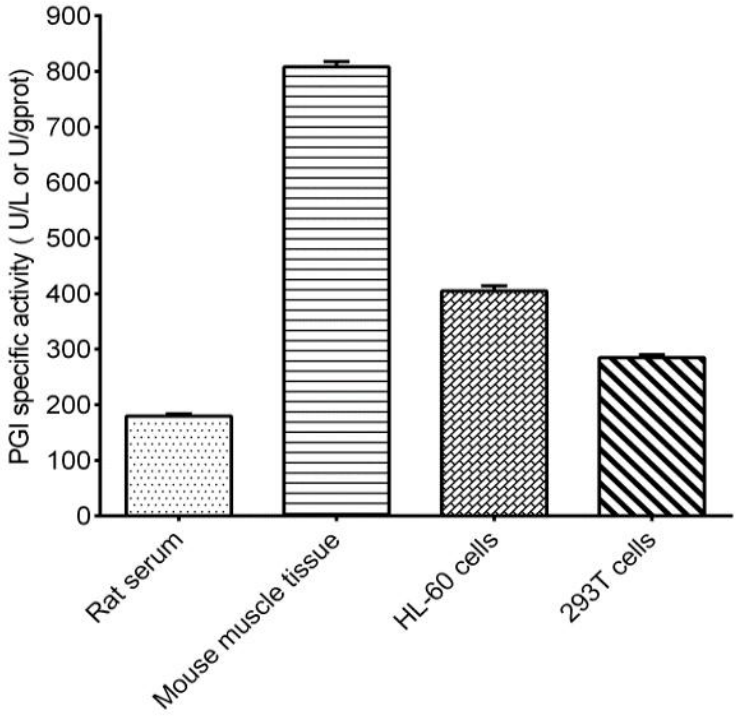
Example analysis:

Take 20 μL of 10% mouse muscle tissue homogenate which was diluted 200 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.8931x - 0.0097, the A_1 value of the sample well is 0.417, the A_2 value of the sample well is 0.962, deltaA_{450} = A_2 - A_1 = 0.962 - 0.417 = 0.545. The protein concentration is 7.33 gprot/L, and the calculation result is:

PGI activity (U/gprot) = (0.545 + 0.0097) ÷ 1.8931 ÷ 10 × 200 ÷ 7.33 × 1000 = 799.5 U/gprot

Detect rat serum (diluted 20 times), 10% mouse muscle tissue homogenate (the protein concentration is 7.33 gprot/L, diluted 200 times), 1.26 x 10^6 HL-60 cells (the protein concentration is 0.76 gprot/L, diluted 10 times) and 1.39 x 10^6 293T cells (the protein concentration is 1.04 gprot/L, diluted 10 times), according to the protocol, the result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.