（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K770-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader(445-460 nm)

Detection range：0.78-50.09 U/L

Elabscience® Phosphoglucose Isomerase (PGI) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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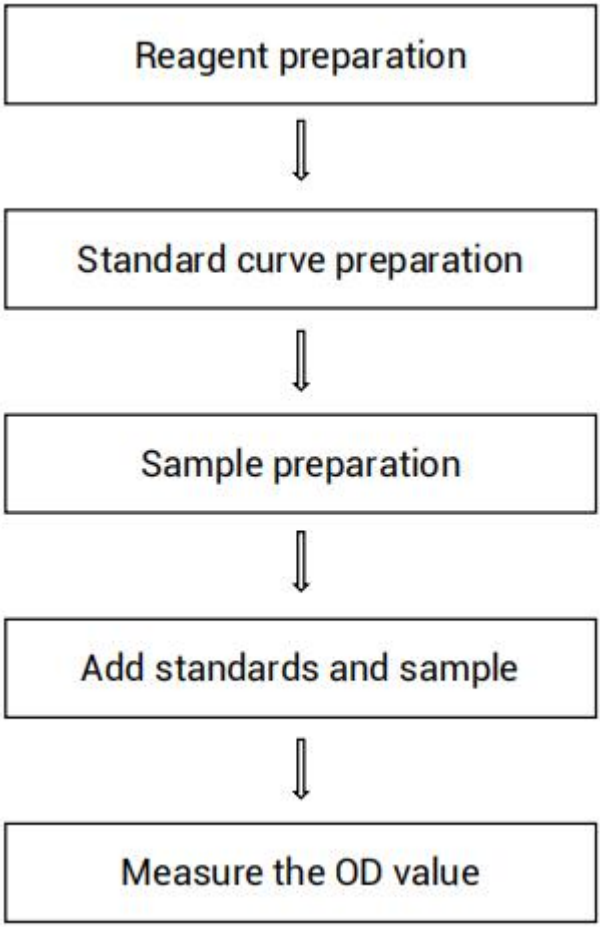
Assay summary



Intended use

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

Detection principle

Phosphoglucose Isomerase (PGI) is an important enzyme that can catalyze 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: The PGI catalyze the substrate to produce the product, reacting with chromogenic agent to form the chromogenic substance, which has the maximum absorption at a wavelength of 450 nm. The PGI enzyme activity is calculated by measuring the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	55 mL × 2 vials	-20℃, 12 months, shading light
Reagent 2	Buffer Solution	55 mL × 1 vial	55 mL × 2 vials	-20℃, 12 months, shading light
Reagent 3	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Oxidant Reagent	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Enzyme Reagent	0.3 mL × 1 vial	0.6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20℃, 12 months, shading light
Reagent 7	5 mmol/L Standard Solution	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months, shading light

	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of measuring working solution:
Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 510 μL of mersuring 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 spot protected from light and used up at the same day.

③ The preparation of 0.5 mmol/L standard solution:
Before testing, please 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 spot protected from light and used up at the same day.

④ The 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① 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℃.

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

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

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

② Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

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

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

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
1×10^6 HL-60 cells	10-20
1×10^6 293T cells	10-20
1×10^6 Jurkat cells	10-20

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

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 A_1. Incubate at 37°C for 10 min and measure the OD value of each well at 450 nm, as A_2. (The standard curve is fitted to the standard well in A_2 value.)

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 absoluted A_2 value.

3. Plot the standard curve by using absoluted 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 hydrolyze the substrate to production 1 μmol of product at 37 °C is defined as 1 unit.

$$\text{PGI activity (U/L)} = (\Delta A_{450} - b) \div a \div T \times f \times 1000$$

2. Tissue samples:

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

$$\text{PGI activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

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

Three human serum were assayed 20 times in duplicate by three operators to determine precision between assays.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	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

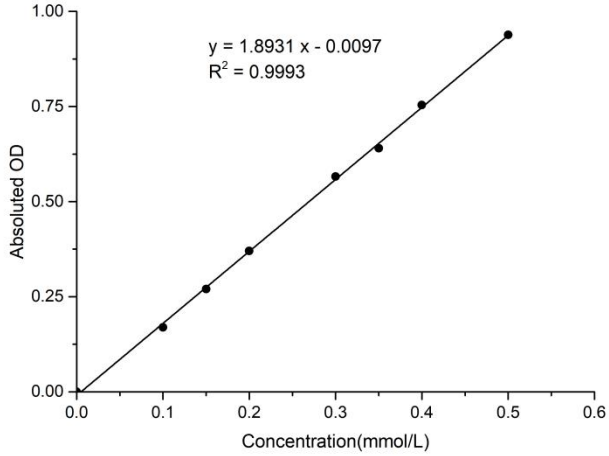
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
A_2 value	0.078	0.244	0.346	0.444	0.644	0.713	0.838	1.013
	0.078	0.251	0.350	0.452	0.644	0.724	0.826	1.020
Average A_2 value	0.078	0.248	0.348	0.448	0.644	0.719	0.832	1.017
Absolute A_2 value	0	0.170	0.270	0.370	0.566	0.641	0.754	0.939



Appendix Π Example Analysis

Example analysis：

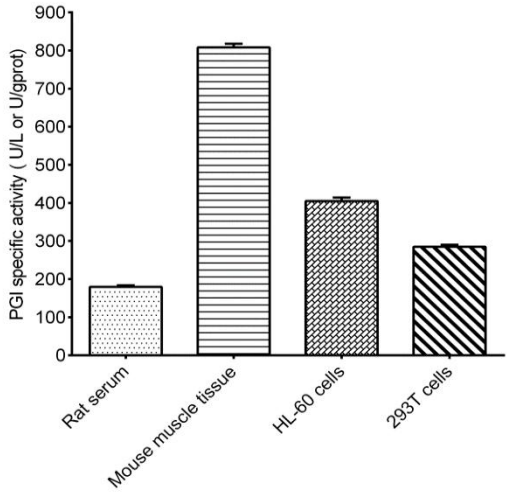
Take 20 μL of 10% mouse muscle tissue homogenate which dilute for 200 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.8931 x - 0.0097, the A_1 value of the sample well is 0.417, the A_2 value of the sample well is 0.962, $\Delta A_{450} = A_2 - A_1 = 0.962 - 0.417 = 0.545$. 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 (dilute for 20 times), 10% mouse muscle tissue homogenate (the protein concentration is 7.33 gprot/L, dilute for 200 times), 1.26×10^6 HL-60 cells (the protein concentration is 0.76 gprot/L, dilute for 10 times) and 1.39×10^6 293T cells (the protein concentration is 1.04 gprot/L, dilute for 10 times), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.