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

Catalog No：E-BC-K1150-M

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

Measuring instrument：Microplate reader(440-460 nm)

Detection range：1.26-101.85 U/L

Elabscience® 6-Phosphogluconate Dehydrogenase (6-PGDH) 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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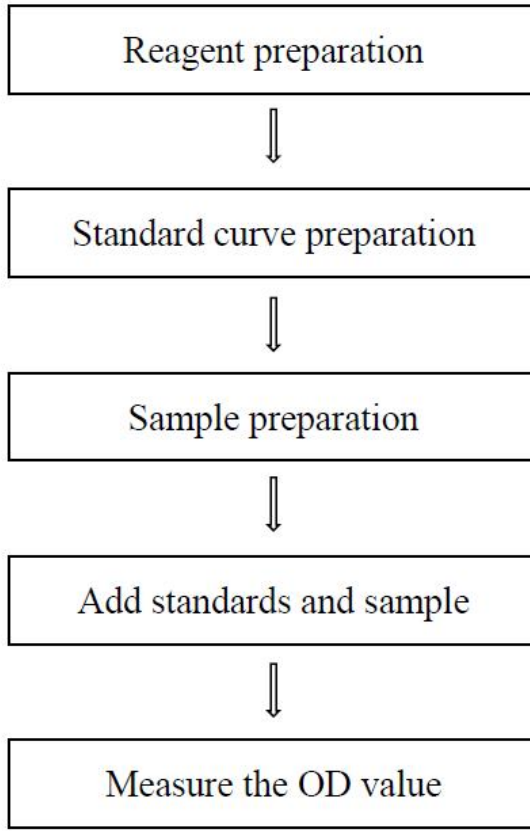
Assay summary



Intended use

This kit can be used to measure the 6-phosphogluconate dehydrogenase (6-PGDH) activity in serum (plasma), plant, animal tissue and cell samples.

Detection principle

6-phosphogluconate dehydrogenase (6-PGDH) is the rate-limiting enzyme of the pentose phosphate pathway, which is responsible for the production of NADPH, energy balance, growth rate, and cell viability. 6-PGDH is also associated with a variety of tumor signaling pathways, including epidermal growth factor signaling pathway and nuclear factor E2 related factor signaling pathway.

The principle of this kit is that the product generated by the 6-PGDH catalytic substrate reacts with the chromogenic agent to generate the chromogenic substance, which has the maximum absorption at 450 nm, and is calculated by measuring the OD value at 450 nm 6-PGDH enzyme activity.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL ×1 vial	-20℃, 12 months shading light
Reagent 2	Substrate	0.55 mL × 1 vial	1.1 mL × 1 vial	-20℃, 12 months shading light
Reagent 3	Coenzyme	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20℃, 12 months shading light
Reagent 5	Standard	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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 (440-460 nm, optimum wavelength: 450 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of coenzyme working solution：

Dissolve coenzyme with 850 μL of double distilled water, mix fully. Aliquoted storage at -20°C for a week protect from light.

③ The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 400 μL of working solution (mix well 250 μL of buffer solution, 20 μL of substrate, 30 μL of coenzyme working solution and 100 μL of chromogenic agent). The working solution should be prepared on spot and store protect from light, and used up on the same day.

④ The preparation of 1000 μmol/L standard solution：

Dissolve standard with 750 μL of double distilled water, mix fully. Aliquoted storage at -20°C for 1 week protect from light.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1000 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 400, 600, 800, 1000 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**50**	**100**	**200**	**400**	**600**	**800**	**1000**
1000 μmol/L standard (μL)	0	10	20	40	80	120	160	200
Double distilled water (μL)	200	190	180	160	120	80	40	0

Sample preparation

Plasma or serum samples: Detect directly.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

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

Cell samples:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection, detect on the same day.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1-10
10% Mouse muscle tissue homogenate	1-10
10% Mouse heart tissue homogenate	1-10
10% Mouse brain tissue homogenate	1-10
10% Mouse kidney tissue homogenate	2-10
10% Broccoli tissue homogenate	1
10% Potato tissue homogenate	1
10% Corn tissue homogenate	1-10
1×10^6 Hela 60 cells	2-10
1×10^6 293T cells	8-25
Rats plasma	1
Fetal bovine plasma	1
Human serum	1

Note: The diluent is PBS(0.01 M，pH 7.4). 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 with different concentrations into the standard wells.

Sample well: Add 20 μL of sample into the sample wells.

② Add 200 μL of working solution into each well.

③ Mix well with microplate reader for 5 s. Measure the OD values of each well at 450 nm with microplate reader, as A_1. Incubate 10 min at 37°C, measure the OD values of each well at 450 nm with microplate reader, as A_2. (The standard curve is calculated as 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. Plasma (serum) samples:

Definition: The amount of 6-PGDH in 1 L of plasma (serum) sample that catalyze decomposition of 1 μmol product at 37 ℃ for 1 min is defined as 1 unit.

$$\text{6-PGDH activity (U/L)} = (\Delta A_{450} - b) \div a \div T \times f$$

2. Tissue sample:

Definition: The amount of 6-PGDH in 1 g of tissue sample that catalyze decomposition of 1 nmol product at 37 ℃ for 1 min is defined as 1 unit.

$$\text{6-PGDH activity (U/g wet weight)} = (\Delta A_{450} - b) \div a \times V_1 \div m \div T \times f \times 1000$$

3. Cell sample:

Definition: The amount of 6-PGDH in 1×10^6 cell sample that catalyze decomposition of 1 nmol product at 37 ℃ for 1 min is defined as 1 unit.

$$\text{6-PGDH activity (U/10^6)} = (\Delta A_{450} - b) \div a \times V_2 \div n \div T \times f \times 1000$$

[Note]

ΔA_{450}: The change value of sample well, $A_2 - A_1$.

T: Reaction time, 10 min

f: Dilution factor of sample before test.

V_1: The volume of tissue homogenate, 0.0009 L

m: The weight of the sample, 0.1 g.

V_2: The volume of cell homogenate, 0.0002 L

n: The number of cells, 1 × 10^6

1000: 1 μmol/L = 1000 nmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples 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)	15.00	25.00	45.00
%CV	2.9	4.6	3.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	15.00	25.00	45.00
%CV	5.9	9.4	6.8

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 100.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	15.00	25.00	45.00
Observed Conc. (U/L)	14.25	26.25	45.90
Recovery rate (%)	95.0	105.0	102.0

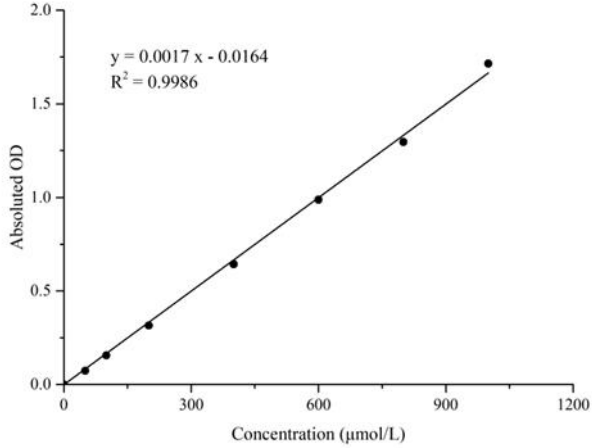
Sensitivity

The analytical sensitivity of the assay is 1.26 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 (μmol/L)	**0**	**50**	**100**	**200**	**400**	**600**	**800**	**1000**
A_2	0.109	0.181	0.263	0.425	0.752	1.094	1.413	1.834
	0.107	0.182	0.265	0.423	0.751	1.098	1.396	1.812
Average A_2	0.108	0.182	0.264	0.424	0.752	1.096	1.405	1.823
Absoluted A_2	0.000	0.074	0.156	0.316	0.644	0.988	1.297	1.715



Appendix II Example Analysis

Example analysis：

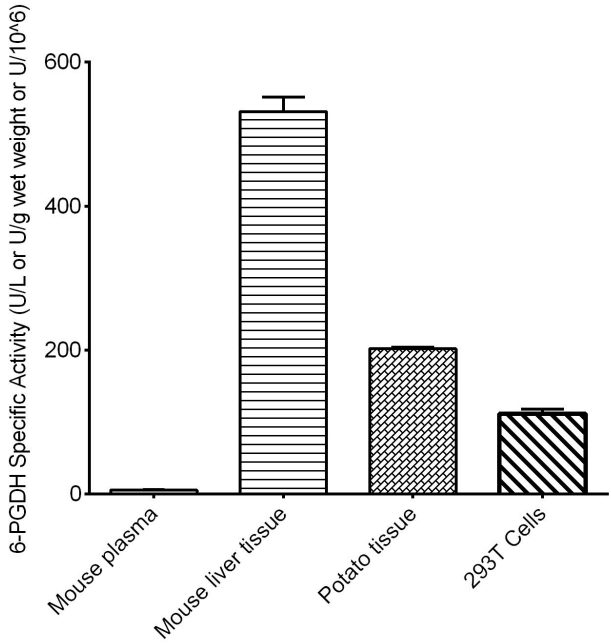
Take 20 μL of 10% mouse kidney tissue homogenate and dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.002x - 0.0117, the A_1 value of the sample well is 0.21, the A_2 value of the sample well is 0.5, $\Delta A_{450} = A_2 - A_1 = 0.5 - 0.21 = 0.29$, and the calculation result is:

6-PGDH activity(U/g wet weight) = (0.29 + 0.0117) ÷ 0.002 × 0.0009 ÷ 0.1 ÷ 10 × 5 × 1000

= 678.83 U/g wet weight

Detect mouse plasma, 10% mouse liver tissue homogenate, potato tissue homogenate and 1×10^6 293Tcells (dilute for 5 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.