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

Catalog No：E-BC-F152

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：6.27-100 μmol/L

Elabscience® 6-Phosphgluconic Acid (6-PGA) Fluorometric 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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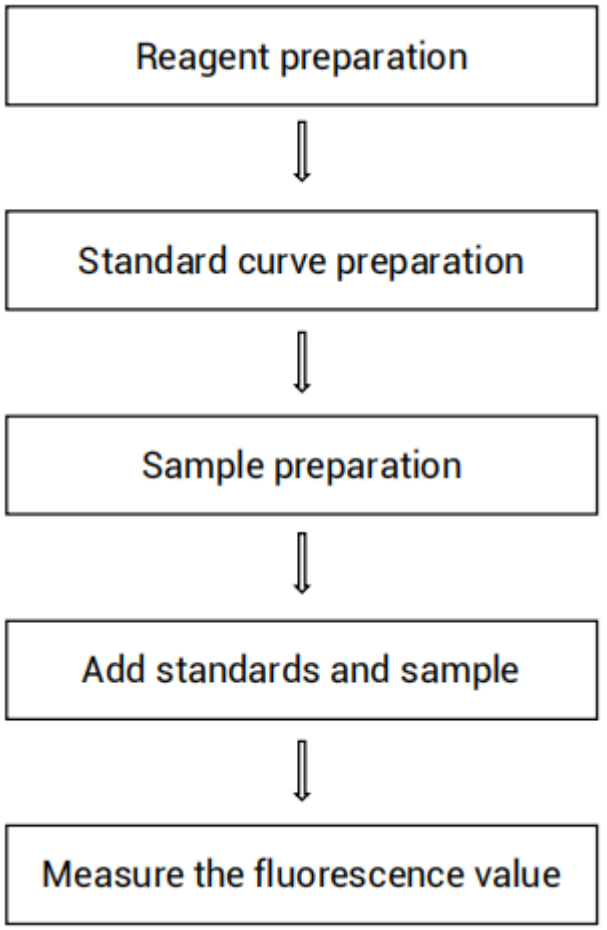
Assay summary



Intended use

This kit can be used to measure 6-phosphgluconic acid (6-PGA) content in animal tissue and cell samples.

Detection principle

6-phosphgluconic acid (6-PGA) is an intermediate in the pentose phosphate pathway. In the pentose phosphate pathway, 6-PGA is converted by 6-phosphogluconate dehydrogenase to ribulose-5-phosphate, which is a precursor for nucleic acid synthesis.

The detection principle of this kit: 6-PGA is converted under enzymatic catalysis, and the fluorescent substrate is transformed into a fluorescent substance. The content of 6-PGA in the sample can be determined by measuring the fluorescence signal of fluorescent substance at an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Substrate	Powder × 6 vials	-20°C, 12 months shading light
Reagent 3	Enzyme Reagent	Powder × 3 vials	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Standard	Powder × 2 vials	-20°C, 12 months shading light
	Black Microplate	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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator

Reagents:

Normal saline (0.9% NaCl)

Material:

10 kDa MWCO Spin Filter (outer tube: 1.5 mL, inner tube: 0.5 mL), double distilled water

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 0.2 mL of double distilled water, mix well to dissolve. Store at -20°C for 2 days protected from light.

③ The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 0.8 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

④ The preparation of chromogenic working solution:
Before testing, please prepare sufficient chromogenic working solution. For example, prepare 500 μL of chromogenic working solution (mix well 5 μL of chromogenic agent and 495 μL of buffer solution). The chromogenic working solution should be prepared on spot protected from light and used up within 8 h.

⑤ The preparation of 100 μmol/L standard:
Dissolve one vial of standard with 10 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

⑥ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 100 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 50, 60, 70, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**50**	**60**	**70**	**100**
100 μmol/L Standard (μL)	0	40	60	80	100	120	140	200
Double distilled water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Homogenize 100 mg tissue in 900 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material.

④ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 10 min at 4°C.

⑤ Take the filtered sample supernatant and detect in the same day.

Cell 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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material.

④ Collect supernatant and add it to 10 kDa MWCO Spin Filter. Centrifuge at 12000×g for 10 min at 4°C.

⑤ Take the filtered sample supernatant and detect in 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% Mouse kidney tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Porcine liver tissue homogenate	1
1×10^6 293T Cells	1
1×10^6 HL-60 Cells	1

Note: The diluent is normal saline (0.9% NaCl). 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 into the wells.
Sample well: add 20 μL of sample into the wells.
Control well: add 20 μL of sample into the wells.

② Add 20 μL of substrate working solution into standard and sample wells. Add 20 μL of double distilled water into control wells.

③ Add 20 μL of enzyme working solution into each well.

④ Add 140 μL of chromogenic working solution into each well.

⑤ Mix fully with fluorescence microplate reader for 5s. Incubate at 37°C for 25 min protected from light. Measure the fluorescence at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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. Tissue samples:

$$\text{6-PGA content (μmol/kg wet weight)} = (\Delta F - b) \div a \times f \div \frac{m}{v}$$

2. Cell samples:

$$\text{6-PGA content (μmol/10^9)} = (\Delta F - b) \div a \times f \div \frac{n}{v}$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{control}$.

f: Dilution factor of sample before tested.

m: The wet weight of tissue, g.

V: The volume of normal saline (0.9% NaCl) during sample preparation, mL.

n: The number of cell sample/10^6.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse lung tissue 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 (μmol/L)	25.0	55.0	75.0
%CV	2.1	3.5	2.8

Inter-assay Precision

Three mouse lung tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	25.0	55.0	75.0
%CV	5.5	8.6	7.5

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc(μmol/L)	25.0	55.0	75.0
Observed Conc(μmol/L)	24.0	55.0	73.5
Recovery rate (%)	96	100	98

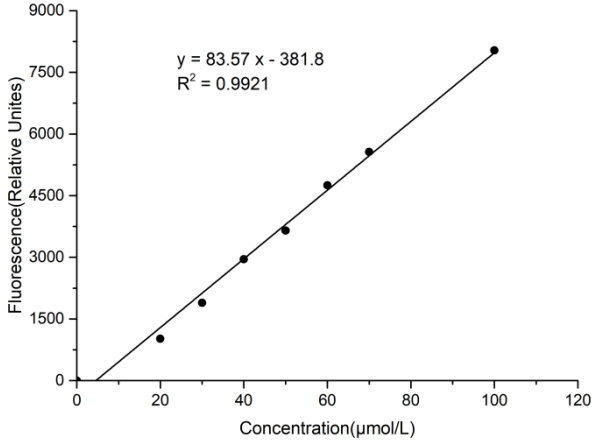
Sensitivity

The analytical sensitivity of the assay is 6.27 μmol/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 fluorescence 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	20	30	40	50	60	70	100
F value	1153	2163	3026	4013	4896	5996	6800	9250
	1353	2379	3264	4397	4910	6016	6834	9328
Average F value	1253	2271	3145	4205	4903	6006	6817	9289
Absoluted F value	0	1018	1892	2952	3650	4753	5564	8036



Appendix Π Example Analysis

Example analysis：

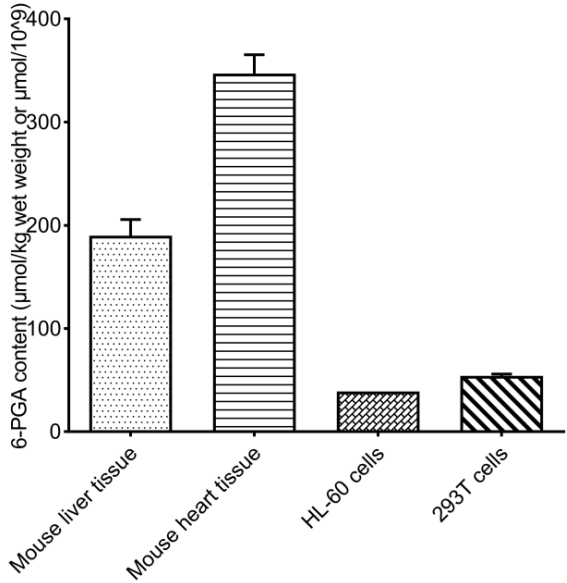
Take 20 μL of 10% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 83.57 x – 381.80, the F value of the sample well is 2362, the F value of the control well is 986, $\Delta F = F_{sample} - F_{control}$ = 2362 - 986 = 1376. The calculation result is:

$$\text{6-PGA content (μmol/kg wet weight)} = (1376 + 381.80) \div 83.57 \div \frac{0.1}{0.9}$$

$$= 189.30 \text{ μmol/kg wet weight}$$

Detect 10% mouse liver tissue homogenate, 10% mouse heart tissue homogenate, 1×10^6 HL-60 cells and 1×10^6 293T cells, 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.