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

Catalog No：E-BC-K611-M

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

Measuring instrument：Microplate reader（330-350 nm）

Detection range：0.08–6.79 U/L

Elabscience®Pyruvate Kinase (PK) Activity 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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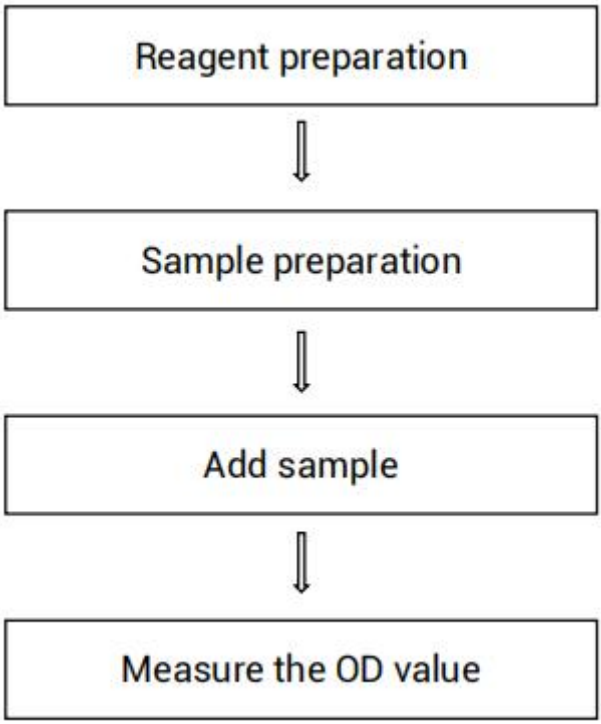
Assay summary



Intended use

This kit can be used to measure pyruvate kinase (PK) activity in serum, plasma, tissue and cell samples.

Detection principle

In the presence of adenosine diphosphate (ADP), pyruvate kinase (PK) catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, Lactate dehydrogenase catalyzes the reaction of pyruvic acid with NADH to produce lactic acid and NAD^+. The activity of pyruvate kinase can be calculated by detection the absorbance changes at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	10 mL × 2 vials	-20℃, 12 months
Reagent 2	Substrate A	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Substrate B	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 4	Enzyme Solution	1.2 mL ×1 vial	1.2 mL × 2 vials	-20℃, 12 months, shading light
	UV 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:

Micropipettor, Vortex mixer, Incubator, Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate A working solution：
Dissolve one vial of substrate A with 1.2 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

③ The preparation of substrate B working solution：
Dissolve one vial of substrate B with 10 mL of buffer solution, mix well to dissolve. Store at -20°C for 3 days protected from light.

④ The preparation of enzyme working solution：
For each well, prepare 40 μL of enzyme working solution (mix well 20 μL of substrate A working solution and 20 μL of enzyme solution). The enzyme working solution should be prepared on spot and used up in the same day.

Sample preparation

① Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

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

⑤ 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 Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

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

⑤ 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
10% Rat kidney tissue homogenate	4-6
10% Rat lung tissue homogenate	4-6
10% Rat liver tissue homogenate	4-6
10% Rat brain tissue homogenate	4-6
10% Mouse liver tissue homogenate	4-6
10% Mouse heart tissue homogenate	4-6
Rat serum	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
Human serum	1
Dog plasma	1
HL-60 cell	1
293T cell	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.

The key points of the assay

① The sample for detection is better to be as fresh as possible, as the activity of PK will be reduced during long-term storage.

② The time of A2 can be extended if the value of serum sample is too low.

Operating steps

① Sample well: Add 10 μL of sample to the wells.

② Add 150 μL of substrate B working solution and 40 μL of enzyme working solution into each well.

③ Measure the OD value of each well at 20 s and 3 min 20 s respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_1 - A_2$.

Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: the enzyme amount of 1 mmol of NADH consumed by 1 L of liquid sample per minute at room temperature is defined as 1 unit

$$\text{PK activity (U/L)} = \left(\frac{\Delta A_{340}}{6220 \times d} \times V_{Total}\right) \div V_{Sample} \div T \times f \times 1000$$

2. Tissue and cells samples:

Unit definition: the enzyme amount of 1 mmol of NADH consumed by 1 g sample protein per minute at room temperature is defined as 1 unit

$$\text{PK activity (U/gprot)} = \left(\frac{\Delta A_{340}}{6220 \times d} \times V_{Total}\right) \div (V_{Sample} \times C_{pr}) \div T \times f \times 1000$$

[Note]

ΔA_{340}: A_2 - A_1.

6220: The molar extinction coefficient of NADH, L/mol•cm

d: Optical path, 0.6 cm.

V_{Total}: The total volume of the reaction system, 0.2 mL.

V_{Sample}: The volume of the sample, 0.01 mL.

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

T: The time of reaction, 3 min.

f: Dilution factor of sample before test.

1000: 1 mol/L=1000 mmol/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)	1.20	3.40	5.80
%CV	5.3	5.0	4.7

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)	1.20	3.40	5.80
%CV	6.8	7.3	6.9

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 103%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	1.8	3.7	6.2
Observed Conc. (U/L)	1.8	3.9	6.5
Recovery rate (%)	99	105	105

Sensitivity

The analytical sensitivity of the assay is 0.08 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.

Appendix Π Example Analysis

Example analysis：

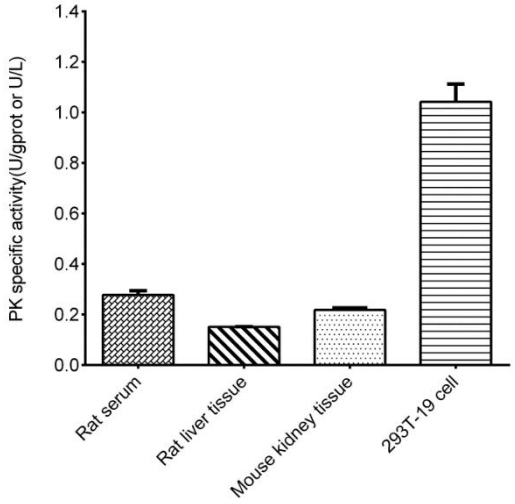
For rat liver tissue, take 10 μL of 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample is 1.043, after 3 minutes of reaction, the A_2 of the sample is 0.892, the concentration of protein in sample is 8.95 gprot/L, and the calculation result is:

$$\text{PK activity (U/gprot)} = (1.043 - 0.892) \div (6220 \times 0.6) \times 0.2 \div (0.01 \times 8.95) \div 3 \times 5 \times 1000$$

$$= 0.15 \text{ U/gprot}$$

Detect rat serum, 10% rat liver tissue homogenate (the concentration of protein is 8.95 gprot/L, dilute for 5 times), 10% mouse kidney tissue homogenate (the concentration of protein is 9.52 gprot/L, dilute for 5 times) and 293T cell (the concentration of protein is 1.80 gprot/L) 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.