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

Catalog No：E-BC-K608-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：6.43-876.53 U/L

Elabscience® Pyruvate Carboxylase (PC) 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：

Phone: 240-252-7368(USA)
Fax: 240-252-7376(USA)
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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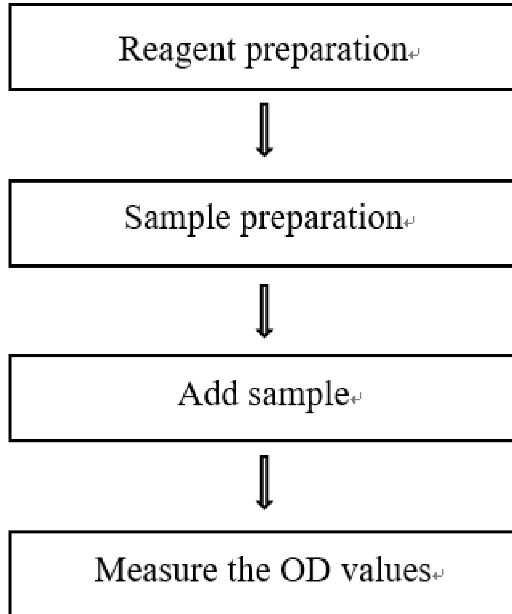
Assay summary



Intended use

The kit can be used to detect the activity of pyruvate carboxylase (PC) in serum, plasma, animal tissue and cell samples.

Detection principle

Pyruvate carboxylate (PC) is a biotin-dependent enzyme that converts pyruvate to oxaloacetic acid by carboxylation. PC is widely found in animals, molds and yeasts, but it is absent from plants and most bacteria. In the tricarboxylic acid cycle, it is the main complementary reaction supplying oxoacetate and the first rate-limiting enzyme in the gluconeogenesis process.
The product of the PC-catalyzed substrate reacts with the reducing agent. The reducing agent has a maximum absorbance at 340 nm, and the enzyme activity of PC is calculated by calculating the amount of reducing agent consumed in the reaction.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL ×1 vial	30 mL ×1 vial	-20℃, 12 months
Reagent 2	Catalyst	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	0.1 mL ×1 vial	0.2 mL ×1 vial	-20℃, 12 months, shading light
Reagent 4	Accelerant	0.1 mL ×1 vial	0.2 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	Reducing Reagent	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
	UV-Microplate	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 (340 nm), Incubator (37°C)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of catalyst working solution：

Dissolve one vial of catalyst with 0.75 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 12 days protected from light.

③ The preparation of reducing working solution：

Dissolve one vial of reducing reagent with 0.5 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 7 days protected from light.

④ The preparation of mersuring working solution：

Before testing, please prepare sufficient working solution according to the sample wells. For example, prepare 200 μL of mersuring working solution (mix well 181 μL of buffer solution, 10 μL of catalyst working solution, 1 μL of enzyme reagent, 1 μL of accelerant and 7 μL of reducing reagent working solution). Keep it on ice during use. The mersuring working solution should be prepared when using and used up within 1 day.

Sample preparation

① Sample preparation:

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

Tissue samples:

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°C.

④ Centrifuge at 10000×g for 10 minutes at 4°C 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°C.

④ Centrifuge at 10000×g for 10 minutes at 4°C 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% Mouse liver tissue homogenate	90-110
10% Mouse kidney tissue homogenate	90-110
10% Mouse heart tissue homogenate	90-110
10% Mouse lung tissue homogenate	70-80
10% Rat liver tissue homogenate	90-110
10% Rat kidney tissue homogenate	90-110
10% Rat heart tissue homogenate	90-110
Rat plasma	10-20
Mouse plasma	10-20
Rat serum	10-20
Mouse serum	10-20
1.92×10^6 CHO cells	2-4
0.99×10^6 293T cells	2-4
1.44×10^6 4T1 cells	2-4
1.58×10^6 Jurkat cells	2-4
1.24×10^6 Molt-4 cells	2-4
0.88×10^6 Hela cells	2-4
1.36×10^6 Raw cells	2-4
1.24×10^6 HL-60 cells	2-4

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 When testing the sample, the value of A_2 should be greater than 0.2, otherwise the sample needs to be diluted before testing.

② The sample reaction rate is fast, and if A_1 is not determined immediately, it may lead to low or no value of the sample determination.

Operating steps

① Sample well: add 10 μL of sample into sample wells.
Blank well: add 10 μL of normal saline (0.9% NaCl) into blank wells.

② Add 190 μL of mersuring working solution to each well.

③ Mix fully for 5 s with microplate reader. After adding the mersuring working solution for 15 s, measure the OD value of each well at 340 nm, recorded as A_1. Incubate at 37°C for 5 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2.

Note: The sample reaction rate is fast, and if A_1 is not determined immediately, it may lead to low or no value of the sample determination.

Calculation

1. **PC activity in serum (plasma) sample**

   Definition: The amount of pyruvate carboxylate (PC) in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol product in 1 minute at 25 ℃ is defined as 1 unit.

$$\text{PC activity (U/L)} = \frac{\Delta A_{340} \times V_1 \times f}{\varepsilon \times d \times V_2 \times T}$$

2. **PC activity in tissue and cell samples**:

   Definition: The amount of pyruvate carboxylate (PC) in 1 g tissue or cell sample that hydrolyze the substrate to produce 1 μmol product in 1 minute at 25°C is defined as 1 unit.

$$\text{PC activity (U/gprot)} = \frac{\Delta A_{340} \times V_1 \times f}{\varepsilon \times d \times V_2 \times T \times C_{pr}}$$

[Note]

ΔA_{blank} : The change OD value of the blank well ($\Delta A_{blank} = A_1 - A_2$).

ΔA_{sample} : The change OD value of the sample well ($\Delta A_{sample} = A_1 - A_2$).

ΔA_{340} : $\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank}$.

ε : The molar extinction coefficient of product at 340nm, 6.22×10^{-3} L/μmol/cm.

d : Optical path, 0.5 cm.

V_1 : The volume of the reaction system, 0.2 mL.

V_2 : The volume of the sample, 10 μL = 0.01 mL.

T: The incubation time, 5 min.

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

f: Dilution factor of the sample before tested.

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)	120.00	328.00	420.00
%CV	0.6	0.8	1.4

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)	120.00	328.00	420.00
%CV	3.9	7.7	9.7

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 100.8%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	120	328	420
Observed Conc. (U/L)	117.6	334.5	436.8
Recovery rate (%)	91	102	109.4

Sensitivity

The analytical sensitivity of the assay is 6.43 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

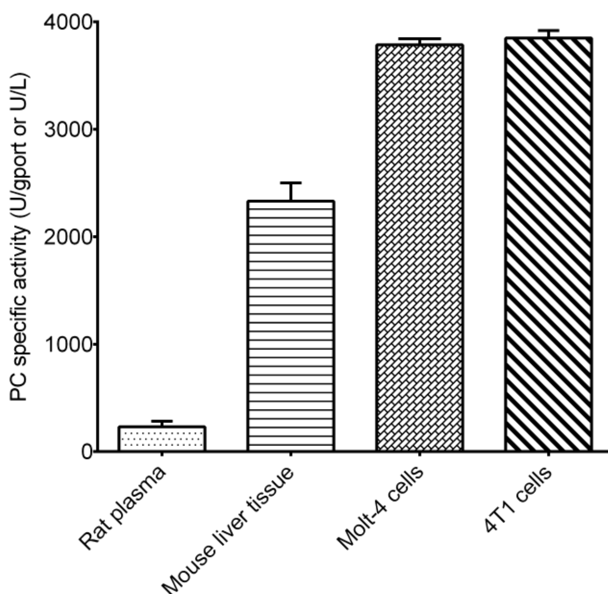
Example analysis:

Dilute 10% mouse liver tissue homogenate, dilute for 100 times, then take 10 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

$\Delta A_{sample} = 0.216$， $\Delta A_{blank} = 0.020$， $\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank} = 0.216 - 0.020 = 0.196$, the concentration of protein in sample is 11.80 gprot/L, and the calculation result is:

$$\text{PC activity (U/gprot)} = \frac{0.196 \times 0.20 \times 100}{6.22 \times 10^{-3} \times 0.5 \times 0.01 \times 5 \times 11.80} = 2136.36 \text{ U/gport}$$

Detect rat plasma, 10% mouse liver tissue homogenate (the concentration of protein is 11.80 gprot/L, dilute for 100 times), 1.24×10^6 Molt-4 cells (the concentration of protein is 0.35 gprot/L) and 1.44×10^6 4T1 cells (the concentration of protein is 0.52 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.