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

Catalog No：E-BC-K605-M

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

Measuring instrument：Microplate reader(340 nm)

Detection range：9.38-231.65 U/L

Elabscience® Phosphoenolpyruvate Carboxykinase (PEPCK) 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

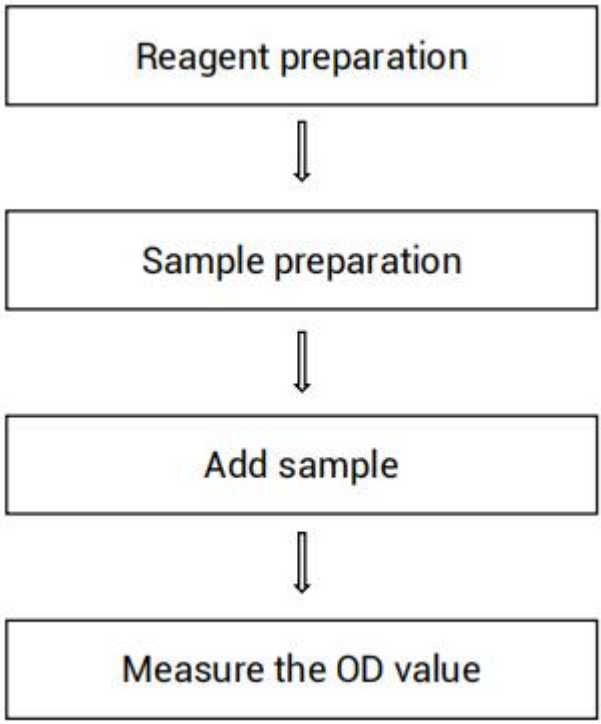
Email: techsupport@elabscience.com

Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

This kit can be used to measure phosphoenolpyruvate carboxykinase (PEPCK) activity in serum (plasma), plant, animal tissue and cell samples.

Detection principle

Phosphoenolpyruvate Carboxykinase (PEPCK) is a key metabolic enzyme that plays a central role in processes such as gluconeogenesis and triglyceride synthesis. As a central regulator for maintaining blood sugar homeostasis and energy metabolism, PEPCK catalyzes the formation of phosphoenolpyruvate (PEP) from oxaloacetic acid, serving as a critical link between carbohydrate metabolism, lipid metabolism, and amino acid metabolism.

The detection principle of this kit: PEPCK catalyzes the substrate reaction and consumes the reducing agent, which has a maximum absorbance at 340 nm. The enzyme activity of PEPCK is calculated by determining the amount of reducing agent consumed per unit time during the reaction.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	55 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	0.25 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Reducing Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Cell Lysis Buffer	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months
	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 (340 nm), Incubator

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 0.5 mL of buffer solution, mix well to dissolve. The substrate working solution should be prepared on spot and protected from light for use. Store at -20°C for 7 days protected from light.

③ The preparation of reducing working solution：
Dissolve one vial of reducing reagent with 0.5 mL of buffer solution, mix well to dissolve. The reducing working solution should be prepared on spot and protected from light for use. Store at -20°C for 7 days protected from light.

④ The preparation of measuring working solution：
Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 5000 μL of measuring working solution (mix well 4850 μL of buffer solution, 40 μL of substrate working solution, 35 μL of enzyme reagent and 75 μL of reducing working solution). The measuring working solution should be prepared on spot and protected from light for use. The prepared solution should be used up within 8 h.

Sample preparation

① Sample preparation

Serum or plasma samples: Detect directly.

Tissue samples:

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

② Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4 °C.

③ Centrifuge at 10000 × g for 10 min at 4 °C 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 is recommended for animal tissue samples. E-BC-K168-M is recommended for plant tissue samples).

Cell samples:

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

② Lyse 1× 10^6 cells with 200 μL cell lysis buffer. Place on the ice box and mix well every 5 min, lyse for 10 min.

③ Centrifuge at 10000 × g for 10 min at 4°C 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 is recommended for cell samples.).

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	100-200
10% Mouse kindey tissue homogenate	100-200
10% Mouse spleen tissue homogenate	50-100
10% Mouse lung tissue homogenate	50-100
10% Corn tissue homogenate	50-100
1×10^6 HL-60 cells	2-5
1×10^6 293Tcells	2-5
1×10^6 Jurkat cells	2-5
Mouse serum	1-3
Mouse plasma	1-3

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

The key points of the assay

During the sample detection process, if the A_2 value of sample well is less than 0.6, the samples need to be diluted before the detection.

Operating steps

① Blank well: Take 10 μL of buffer solution into the wells.
Sample well: Take 10 μL of sample into the wells.

② Add 200 μL of measuring working solution into blank wells and sample wells.

③ Mix fully for 5 s with microplate reader and measure the OD value of each well at 340 nm as A_1. Incubate at 37°C for 3 min, measure the OD value of each well at 340 nm as A_2.

Calculation

① **The serum and plasma sample**:

Definition: The amount of enzyme in 1 L serum (plasma) per minute that catalyze substrate to product 1 μmol production at 37°C is defined as 1 unit.

$$\text{PEPCK activity (U/L)} = \frac{\Delta A_{340} \times V_{total} \times f}{\varepsilon \times d \times V_{sample} \times T}$$

② **The tissue or cell sample**:

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

$$\text{PEPCK activity (U/gprot)} = \frac{\Delta A_{340} \times V_{total} \times f}{\varepsilon \times d \times V_{sample} \times T \times C_{pr}}$$

[Note]

ΔA_{340}: The change OD value of sample well – the change OD value of blank well ($\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank}$)

ΔA_{blank}: The change OD value of blank well ($\Delta A_{blank} = A_{1\,blank} - A_{2\,blank}$)

ΔA_{sample}: The change OD value of sample well ($\Delta A_{sample} = A_{1\,sample} - A_{2\,sample}$)

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

d: Optical path, 0.60 cm.

V_{total}: The volume of reaction system, 0.21 mL

V_{sample}: The volume of the sample added to the reaction system, 0.01mL

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

f: Dilution factor of sample before tested.

T: Reaction time, 3 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse 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)	50.00	100.00	150.00
%CV	4.0	5.0	4.3

Inter-assay Precision

Three mouse 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)	50.00	100.00	150.00
%CV	4.8	7.3	8.3

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 97%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	50.00	100.00	150.00
Observed Conc. (U/L)	50.0	96.0	142.5
Recovery rate (%)	100.0	96.0	95.0

Sensitivity

The analytical sensitivity of the assay is 9.38 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

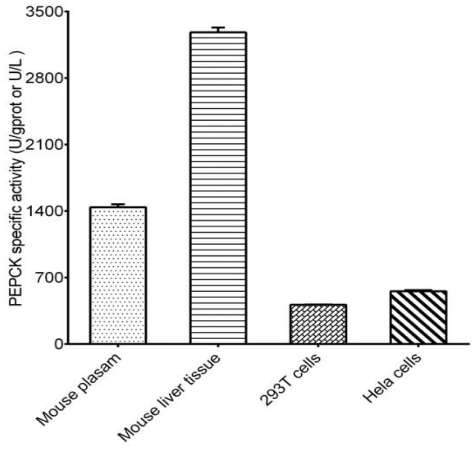
Example analysis：

Take 10 μL of 10% mouse liver tissue homogenate (dilute for 200 times) and carry the assay according to the operation table. The results are as follows: The A_1 value of the sample well is 0.858, the A_2 value of the sample well is 0.678, $\Delta A_{sample} = A_{1sample} - A_{2sample} = 0.858 - 0.678 = 0.180$, the A_1 value of the blank well is 1.036, the A_2 value of the blank well is 0.986,

$\Delta A_{blank} = A_{1blank} - A_{2blank} = 1.036 - 0.986 = 0.050$, $\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank} = 0.180 - 0.050 = 0.130$, the concentration of protein in sample is 14.5 gprot/L and the calculation result is:

$$\text{PGK activity (U/gprot)} = \frac{0.130 \times 0.21 \times 200}{(6.22 \times 10\text{^-3}) \times 0.6 \times 0.01 \times 3 \times 14.5} = 3363.27 \text{ U/gprot}$$

Detect mouse plasma (dilute for 2 times),10% mouse liver tissue homogenate (the concentration of protein is 14.5 gprot/L, dilute for 200 times), 1×10^6 293T cells (the concentration of protein is 2.93 gprot/L, dilute for 2 times), 1×10^6 Hela cells (the concentration of protein is 1.78 gprot/L, dilute for 2 times) 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.