

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

Phosphoenolpyruvate CarboxyKinase (PEPCK) Activity Assay Kit

Catalog No.: BC00133

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Phosphoenolpyruvate CarboxyKinase Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, cells, tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Ultraviolet spectrophotometer (340 nm)

Product Introduction

Phosphoenolpyruvate carboxykinase (PEPCK) is widely found in animals, plants, microorganisms, and cultured cells. It catalyzes the reaction of oxaloacetate to produce phosphoenolpyruvate and carbon dioxide and is a key enzyme regulating gluconeogenesis.

Principle

PEPCK catalyzes the reaction of oxaloacetic acid to produce phosphoenolpyruvate and carbon dioxide. Pyruvate kinase and lactate dehydrogenase further catalyze the conversion of NADH to NAD⁺. The rate of NADH reduction is measured at 340 nm to calculate PEPCK activity.

Components

Product Name	Size	Storage
Extract	60 mL	Store at 4°C
Reagent 1	Liquid 45 mL × 1 bottle	Store at 4°C
Reagent 2	Liquid 41 µL × 1 bottle	Store at 4°C
Reagent 3	Powder × 1 tube	Store at -20°C
Reagent 4	Powder × 1 tube	Store at -20°C

Note: 1. Dissolve the reagent in 2.5 mL of distilled water before use; store any unused reagent at 4°C for one week.

2. Preparation of working solution: Before use, transfer reagent 2 and reagent 3 to reagent 1 and mix to dissolve. Store any unused reagents at 4°C for one week.

Storage

Store this kit according to the specified conditions upon receipt. The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Bacteria or cultured cells: First, collect bacteria or cells into centrifuge tubes, centrifuge, and discard the supernatant; according to the ratio of bacteria or cell number (10^4) : extraction liquid volume (mL) of 500-1000:1 (it is recommended to add 1 mL of extraction liquid for 5 million bacteria or cells), sonicate the bacteria or cells (ice bath, power 20% or 200W, sonicate for 3 seconds, with 10-second intervals, repeat 30 times); centrifuge at 8000g for 10 minutes at 4°C, collect the supernatant, and place it on ice for testing.
2. Tissue: Homogenize the tissue at a ratio of 1:5~10 (approximately 0.1g of tissue to 1mL of extraction solution is recommended) in an ice bath. Centrifuge at 8000g, 4°C for 10min, and collect the supernatant on ice for analysis.
3. Serum (plasma) samples: direct detection

Operation process

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 340 nm, and zero it with distilled water.
2. Preheat the working solution and reagent four to 37°C (mammals) or 25°C (other species) for 5 minutes.

Reagents	Test tube
Sample (μL)	50
Reagent 4 Working Solution (μL)	50
Working solution (μL)	900
Add the above reagents to a 1 mL quartz cuvette in sequence, mix immediately, start timing at the same time as adding the sample, record the initial absorbance A1 at a wavelength of 340 nm and the absorbance A2 after 1 minute, and calculate $\Delta A = A1 - A2$.	

Note: Before conducting the formal test, please perform 1-2 preliminary experiments. If $\Delta A > 0.1$, the sample needs to be diluted appropriately with the extract before measurement to make $\Delta A < 0.1$, which can improve the detection sensitivity. Multiply by the corresponding dilution factor in the calculation formula.

Result calculation

1. Calculation of serum (plasma) activity:

Unit definition: One unit of enzyme activity is defined as the amount of NADH consumed per minute per mL of serum (plasma).

$$\text{PEPCK (U/mL)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div \text{Cpr} \times V_{\text{sample}} \div T = 3215 \times \Delta A$$

2. Calculation of PFK activity in tissues, bacteria, or cells:

(1) Calculated based on sample protein concentration

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH consumed per minute per mg of tissue protein.

$$\text{PEPCK (U/mg)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (\text{Cpr} \times V_{\text{sample}}) \div T = 3215 \times \Delta A \div \text{Cpr}$$

(2) Calculated based on sample fresh weight

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH consumed per minute per gram of tissue.

$$\begin{aligned} \text{PEPCK (U/mg)} &= [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T \\ &= 3215 \times \Delta A \div \text{Cpr} \end{aligned}$$

(3) Calculated by bacterial or cell density

Definition of a unit: One unit of enzyme activity is defined as 1 nmol of NADH consumed per minute by 10,000 bacteria or cells.

$$\text{PEPCK (U/10}^4 \text{ cells)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (500 \times V_{\text{sample}} \div V_{\text{total sample}}) \div T$$
$$= 3215 \times \Delta A \div \text{Cpr}$$

Annotation

ϵ : The molar absorptivity of NADH at 340 nm is 6.22×10^3 L/mol/cm;

d: Cuvette path length (cm), 1cm;

$V_{\text{total reaction}}$: Total volume of the reaction system (L), $1000 \mu\text{L} = 1.00 \times 10^{-3}$ L;

10^9 : $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$;

Cpr: Protein concentration in the supernatant (mg/mL)

V_{sample} : Volume of supernatant added to the reaction system (mL), $50 \mu\text{L} = 0.05 \text{ mL}$;

$V_{\text{total sample}}$: Add the extraction solution volume, 1 mL

T: Catalytic reaction time (min), 5 min.

W : Sample quality (g)

500: Total number of bacteria or cells, 5 million.

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.