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CheKine™ Micro Phosphoenolpyruvate Carboxylate (PEPC) Activity Assay Kit

Cat #: KTB1122 Size: 48 T/96 T

	Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit		
REF	**Cat #:** KTB1122	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Phosphoenolpyruvate Carboxylase (PEPC) (EC 4.1.1.31) is widely present in animals, plants, microorganisms and cells. It is an enzyme that catalyzes the reaction of phosphoenolpyruvic acid with Carbon Dioxide to produce oxaloacetic acid and presents an irreversible reaction. The operation of the acid cycle plays an important regulatory role. CheKine™ Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit provides a simple method for detecting Phosphoenolpyruvate Carboxylase (PEPC) (EC 4.1.1.31) activity in a variety of biological samples such as Serum, Plasma, Animal Tissues, Plant Tissues, Cells, and Bacteria. In the assay, Phosphoenolpyruvate carboxylase (PEPC) catalyzes phosphoenolpyruvate and CO_2 to produce oxaloacetate and HPO_4^{2-}, and malate dehydrogenase further catalyzes oxaloacetate and NADH to produce malate and NAD^+. The rate of NADH decrease at 340 nm can reflect Phosphoenolpyruvate carboxylase (PEPC) activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	8 mL	16 mL	4℃
Reagent Ⅱ	0.85 mL	1.7 mL	-20℃, protected from light
Reagent Ⅲ Stock Solution	5 μL	10 μL	4℃, protected from light
Reagent Ⅲ Buffer	2.5 mL	5 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Incubator
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Freezing centrifuge

• Deionized water
• Dounce homogenizer(for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working Reagent Ⅱ: Prepared before use, dilute Reagent Ⅱ 10 times with Reagent Ⅰ, that is, take 1 volume of Reagent Ⅱ and add 9 times volume of Reagent Ⅰ. Place in 37℃(mammals) or 25℃(other species) water bath for 5 min.
Working Reagent Ⅲ: Prepared before use, dilute Reagent Ⅲ Stock Solution 400 times with Reagent Ⅲ Buffer, that is, take 1 volume of Reagent Ⅲ Stock Solution and add 399 times volume of Reagent Ⅲ Buffer.

Sample Preparation

1. Serum, Plasma: Tested directly.
2. Animal Tissues: Homogenize tissue at 1 mL/0.1 g in Extraction Buffer. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, place it on ice to be tested.
3. Cells or Bacteria: Collect appropriate number of cells or bacteria for each assay, discard the supernatant. Add 1 mL Extraction Buffer for every 5×10^6 cells or bacteria. Ultrasonic wave breaks cells or bacteria (ice bath, power 20% or 200 W, ultrasonic wave 3 s, interval 10 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, place it on ice to be tested.
4. Plant Tissue Samples: Homogenize tissue at 1 mL/0.1 g in Extraction Buffer, and Ultrasonic wave breaks (ice bath, power 20% or 200 W, ultrasonic wave 3 s, interval 10 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, place it on ice to be tested.

Note: For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet spectrophotometer was returned to zero with deionized wate.
2. Add 10 μL Sample, 20 μL Working Reagent Ⅲ, 170 μL Working Reagent Ⅱ in a 96-well UV Plate or microquartz cuvette. Mix well and immediately measure at 340 nm to read the absorbance. The absorbance value of 20 s is recorded as A_1, 5 min 20 s is A_2, $\Delta A= A_1-A_2$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. It is need to use UV microplate.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. Calculation formulae based on 96-well UV plates are as below:
1. Calculation of PEPC activity in serum (plasma)
Active unit definition: One unit defines as the amount of enzyme that catalyzes and consumes 1 nmol NADH per min in 1 mL Serum (Plasma).
PEPC (U/mL)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\div T$**=1,286×ΔA**
2. Calculation of PEPC activity in tissues, bacteria or cells
(1) Calculated by protein concentration
Active unit definition: One unit defines as the amount of enzyme that catalyzes and consumes 1 nmol NADH per min per mg of

sample.

PEPC (U/mg prot)=[ΔA×V_{total} ÷(ε×d)×10^9]÷(Cpr×V_{sample}) ÷T**=1,286×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: One unit defines as the amount of enzyme that catalyzes and consumes 1 nmol NADH per min per g of Sample.

PEPC (U/g fresh weight)=[ΔA×V_{total} ÷(ε×d)×10^9]÷(V_{sample}÷$V_{sample\ total}$×W)÷T**=1,286×ΔA÷W**

(3) Calculated by bacteria or cells numbers

Active unit definition: One unit defines as the amount of enzyme that catalyzes and consumes 1 nmol NADH per min per 10^4 of Sample number.

PEPC (U/10^4)=[ΔA×V_{total} ÷(ε×d)×10^9]÷(V_{sample}÷$V_{sample\ total}$ × 500)÷T**=2.572×ΔA**

Where: ε: NADPH molar extinction coefficient 6.22×10^3 L/mol/cm; V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.01 mL; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL; T: reaction time, 5 min; Cpr: sample protein concentration, mg/mL; W: sample weight, g; d: 96-well UV plate light path, 0.5 cm; 500: Total number of bacteria or cells, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.