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CheKine™ Micro Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit

Cat #: KTB1126 Size: 48 T/96 T

	Micro Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit		
REF	**Cat #:** KTB1126	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissue, Plant Tissue, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

PEPCK (EC 4.1.1.32) is widely found in animals, plants, microorganisms and cells and catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, a key enzyme in the regulation of gluconeogenic pathways. CheKine™ Micro Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit provides a simple, convenient and rapid method for PEPCK activity detection, which is suitable for testing animal and plant tissues, cells, bacteria, serum (plasma) and other samples. The principle is that PEPCK catalyzes the oxidation of oxaloacetate to produce phosphoenolpyruvate and CO_2, pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to produce NAD^+ in turn, The rate of NADH decline was measured at 340 nm, which reflects PEPCK activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	9 mL	18 mL	4℃
Reagent Ⅱ	8.2 μL	16.4 μL	-20℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	18 μL	36 μL	-20℃, protected from light
Reagent Ⅴ	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, water bath
- Deionized water

• 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 Solution: Prepared before use. Reagent Ⅱ, Reagent Ⅲ and Reagent Ⅳ were transferred to Reagent Ⅰ to dissolve for use. Unused reagents can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent Ⅴ: Prepared before use. add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. Unused reagents can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Serum (Plasma): Direct detection.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

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 water.
2. Working Solution and Working Reagent Ⅴ incubate for 5 min at 37℃ (mammal) or 25℃ (other species).
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Test Well（μL）
Sample	10
Working Solution	180
Working Reagent Ⅴ	10

4. After mixing quickly, record the absorbance values of 20 s and 1 min 20 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A_{Test}=A_1-A_2$.

Note: Blank well only needs to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

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. 96-well UV plates calculation formula

1. Calculation of PEPCK activity in serum

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum per min.

PEPCK (U/mL)=[$\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div V_{Sample}\div T$**=6,430.87×$\Delta A_{Test}$**

2. Calculation of PEPCK activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

PEPCK (U/mg prot)=[$\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**=6,430.87×$\Delta A_{Test}$÷Cpr**

(2) Calculation according to the weight of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min.

PEPCK (U/g fresh weight)=[$\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extraction\ Buffer}\times V_{Sample})\div T$**=6,430.87×$\Delta A_{Test}$÷W**

3. Calculation of PEPCK activity in cells or bacteria

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 cells or bacteria per min.

PEPCK (U/10^4)=[$\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Extraction\ Buffer}\times 500)\div T$**=12.86×$\Delta A_{Test}$**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L, $V_{Extraction\ Buffer}$: the volume of the Extraction Buffer, 1 mL; V_{Sample}: the volume of the supernatant in the reaction system, 0.01 mL; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 1 min; W: sample weight, g; 500: total number of cells or bacteria, 5 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

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
KTB3021	CheKine™ Micro NAD-Malate Dehydrogenase (NAD-MDH) Activity Assay Kit
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit

Disclaimer

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