Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0393

Size:100T/96S

Components:

Extract solution: 110 mL ×1. Storage at 4℃.

Reagent I: 18 mL×1, stored at 4℃.

Reagent II: Powder×1, stored at -20℃ and protected from light. Dissolve it in 15 mL of Reagent I before use. The reagent that cannot be used up shall be stored at -20℃ after repacking, repeat freezing and thawing are prohibited.

Reagent III: 18 μL×1, stored at 4℃ and protected from light. Before temporary use, add distilled water to dilute according to the volume ratio of 1:120, prepare when the solution will be used.

Reagent IV: 62 μL×1, stored at 4℃ and protected from light. Before temporary use, add distilled water to dilute according to the volume ratio of 7:250, prepare when the solution will be used.

Reagent V： Powder×1, stored at -20℃ and protected from light. Dissolve it in 2.5 mL of distilled water before use. The reagent that cannot be used up shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Product Description:

PEPCK (EC 4.1.1.32) is widely found in animals, flowering plants, algae, some fungi and bacteria. The enzyme catalyzes the conversion of oxaloacetic acid to phosphoenolpyruvate, which is the first-rate limiting enzyme regulating gluconeogenesis.

PEPCK catalyzes oxaloacetic acid to form phosphoenolpyruvate and CO_2, pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to NAD^+ in turn, and determine the NADH decline rate at 340 nm, which can reflect the PEPCK activity.

Reagents and Equipment Required but Not Provided

Ultraviolet spectrophotometer/microplate reader, low temperature centrifuge, water bath pot, micro quartz cuvette/96 well flat-bottom plate (UV), adjustable pipette, mortar/homogenizer, ice and distilled water

Procedure

I. Extraction of crude enzyme solution:

1. Tissue sample:

The proportion of tissue mass (g): volume of extract solution (mL): 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of extract solution) for ice bath homogenate, then centrifugate at 8000 ×g for 10 minutes at 4℃, take the supernatant, place it on ice for testing.

2. Cell sample:

First, collect bacteria or cells into the centrifuge tube, and then discard the supernatant; the number of cells (10^4): the volume of the extract solution (mL) is 500-1000:1 (1 mL of the extract solution is recommended to be added to 5 million cells), and the cells are broken by ultrasonic wave in ice bath (Power: 200W or 20%, ultrasonic:3s, interval:10s, Repeat 30 times). Then centrifuged at 8000 ×g for 10 minutes at 4℃, and the supernatant is taken for test.

3. Serum sample: direct determination.

1. Test procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm, and adjust to zero with distilled water.

2. Working solution: mix Reagent II, Reagent III, Reagent IV in the proportion of 7:1:1(V:V:V) before use. Prepare when the solution will be used.

3. Preheat the working solution at 37℃ (mammal) or 25℃ (other species) for 5 minutes.

4. Operation table: Add the following reagents to the micro quartz cuvette/96 well plate (UV) in turn：

Reagent name (μL)	Blank tube(B)	Test tube(T)
Sample	-	10
Distilled water	10	-
Working solution	180	180
Reagent V	10	10
Add Reagent V and mix it immediately. Measure the absorbance value A1 at 340 nm for 10s and A2 at 70s. Calculate $\Delta A_T = A_{1T} - A_{2T}$, $\Delta A_B = A_{1B} - A_{2B}$, and $\Delta A = \Delta A_T - \Delta A_B$.		

III. Calculation of PEPCK:

1. Calculation by micro quartz cuvette

(1) Calculated by tissue protein concentration:

Definition of enzyme activity: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADH per minute every milligram of protein.

PEPCK activity (U/mg prot) = $\Delta A \div (\varepsilon \times d) \times V_{RT} \times 10^9 \div (V_S \times Cpr) \div T = 3215.4 \times \Delta A \div Cpr$

(2) Calculated by the quality of tissue samples:

Definition of enzyme activity: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADH per minute every gram of sample.

PEPCK activity (U/g fresh weight) = $\Delta A \div (\varepsilon \times d) \times V_{RT} \times 10^9 \div (V_S \div V_{ST} \times W) \div T = 3215.4 \times \Delta A \div W$

(3) By cell count:

Definition of enzyme activity: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADH per minute every 10 thousand cells

PEPCK activity ($U/10^4$ cell)=$\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \div V_{ST} \times 500) \div T = 6.43 \times \Delta A$

(4) Calculated by serum volume:

Definition of enzyme activity: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADH per minute every milliliter of serum

PEPCK activity (U/mL) = $\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div V_S \div T = 3215.4 \times \Delta A$

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;
d: Light diameter of cuvette, 1 cm;
V_{RT}: Total volume of reaction system, 2×10^{-4} L;
V_S: The volume of sample in reaction system, 0.01 mL;
V_{ST}: The volume of extract solution, 1 mL;
Cpr: Sample protein concentration, mg/mL, Self-determination of protein concentration;
W: The mass of sample mass, g;
T: Reaction time, 1 minute;
500: Total number of bacteria or cells, 5 million;
10^9: Unit conversion factor, 1 mol = 10^9 nmol.

2. Calculated by 96 well (UV) plate

Change the d=1 cm in the above formula to 0.6 cm (96 well plate optical diameter) for calculation.

Note:

1. When A1 is less than 1 or ΔA is greater than 0.6 (96 well UV plate is when A1 is less than 0.6 or ΔA is greater than 0.4), it is recommended to dilute the sample to a proper multiple before determination to improve the detection sensitivity.
2. For samples with high enzyme activity, such as animal liver, kidney and other tissues, it is recommended to dilute the extract to 5 times or more for determination.
3. The blank tube is a test well for testing the quality of each reagent component. Under normal conditions, the change does not exceed 0.06.
4. The steps of sample adding and mixing shall be rapid, and the stopwatch timing shall be accurate.

Experimental Example:

1. Take 0.1g liver and add 1 ml extract solution for homogenate. Take the supernatant and dilute it twice with the extract solution. Then operate according to the determination steps. Use a micro quartz cuvette to measure and calculate: $\Delta A_T = A1_D - A2_D = 1.1298-0.4464 = 0.6834$, $\Delta A_B = A1_B - A2_B = 1.3819-1.3463 = 0.0356$, $\Delta A = \Delta A_T - \Delta A_B = 0.6834-0.0356 = 0.6478$

PEPCK activity (U/g mass) = $3215.4 \times \Delta A \div W \times 2$ (dilution ratio) = $3215.4 \times 0.6478 \div 0.1 \times 2 = 41658.72$ U/g mass.

2. Take 0.1g aloe vera and add 1 ml extract solution for homogenization, take the supernatant and operate according to the determination steps. Measure with micro quartz cuvette and calculate $\Delta A_T = A1_T - A2_T = 1.4015-1.2665 = 0.135$, $\Delta A_B = A1_B - A2_B = 1.3819-1.3463 = 0.0356$, $\Delta A = \Delta A_T - \Delta A_B = 0.135-0.0356 = 0.0994$

PEPCK enzyme activity (U/g mass) = $3215.4 \times \Delta A \div W = 3215.4 \times 0.0994 \div 0.1 = 3196.108$ U/g mass.

Related Products:

NA0810/NA0568 Pyruvate Carboxylase PC) Activity Assay Kit
NA0801/NA0560 Fructose 1,6-bisphosphatase FBP) Activity Assay Kit