Phosphoenolpyruvate carboxylase (PEPC) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0474

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃;

Reagent I: Liquid 15 mL×1, store at 4℃;

Reagent II: Liquid 2 mL×1, store at 4℃.

Reagent III: Liquid 2 mL×1, store at 4℃.

Reagent IV: Powder ×1, store at -20℃. Add 2 mL of double distilled water before use; Mix thoroughly. It can be stored at -20℃ after sub packaging. Avoid repeated freezing and thawing;

Reagent V: Powder ×1, store at -20℃. Add 2 mL of double distilled water before use; Mix thoroughly. It can be stored at -20℃ after sub packaging. Avoid repeated freezing and thawing;

Reagent VI original solution: Liquid 10 μL×1, store at 4℃;

Reagent VI diluent: Liquid 5 mL×1, store at 4℃;

Reagent VII: Powder ×1, store at -20℃. Add 5 mL of double distilled water before use; Mix thoroughly. It can be stored at -20℃ after sub packaging. Avoid repeated freezing and thawing;

Reagent VI working solution: Dilute the original solution of reagent VI: diluent of reagent VI in the proportion of 1.6 μL: 328.4 μL. Match the solution as much as you use.

Product Description:

Phosphoenolpyruvate carboxylase (EC 4.1.1.31) is widely found in plants and microorganisms. It catalyzes the irreversible reaction of phosphoenolpyruvate and carbon dioxide to oxaloacetic acid. It is also a key enzyme for C4 plants and cam plants to fix CO_2. It plays an important role in regulating the operation of tricarboxylic acid cycle.

PEPC can catalyze phosphoenolpyruvate and carbon dioxide to form oxaloacetate and HPO_4^{2-}. Malate dehydrogenase can catalyze oxaloacetate and NADH to produce malate and NAD^+. The rate of NADH reduction was measured at 340 nm and PEPC activity was calculated.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well UV plate, pipette, mortar/homogenizer, ice and distilled water.

Protocol

I. Preparation:

1. Tissue: according to the tissue weight (g): the Extract solution volume (mL) is 1:5-10. (It is

recommended that add 1 mL of Extract solution to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 8000 g for 20 minutes at 4℃. Take the supernatant for test.

2. Cells: according to the number of the cells (10^4): the volume of the Extract solution (mL) is 500~1000:1. It is suggested that add 1 mL of Extract solution to 500 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 8000 g 4℃ for 20 minutes. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Working solution: According to the volume ratio of Reagent II, Reagent III, Reagent IV, Reagent V, Reagent VI working solution, Reagent VII = 15: 15: 15: 15: 19: 19 to mix thoroughly. Prepare when the solution will be used.

3. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	90	90
Working solution	90	90
Sample	20	-
Distilled water	-	20

The above reagents are added into the micro quartz cuvette/96 well UV plate in sequence. Mix thoroughly. Measure the absorbance A1 at 340 nm for 10s. Quickly placed in a 30℃ water bath or incubator for 5 min (If the microplate reader has a temperature control function to adjust the temperature to 30℃). Take it out and dry it quickly. Measurement of absorbance A2 at 310s. $\Delta A_B=A1_B-A2_B$. $\Delta A_T=A1_T-A2_T$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only need to test once or twice.

III. PEPC Calculation:

a. Micro quartz cuvette

1. Tissue

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of 1 mg tissue protein that catalyzes the consumes of 1 nmol NADH per minute in the reaction system.

PEPC (U/mg prot)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_S)\div T\times 10^9$= $321\times\Delta A\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every g sample.

PEPC (U/g weight)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_{ST}\times V_S)\div T\times 10^9=321\times\Delta A\div W$

2. Cells or germ

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every 10^4 cells or germ.

PEPC (U/10^4 cell)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\div(V_S\div V_{ST}\times cells(million))\div T\times 10^9=321\times\Delta A\div cells(million)$

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
V_{RT}: Total reaction volume, 0.0002 L;
V_S: Sample volume, 0.02 mL;
Cpr: Protein concentration, mg/mL;
W: Sample weight, g;
V_{ST}: Extract solution volume of cells, 1 mL;
T: Reaction time, 5 min;
10^9: Unit conversion factor,1 mol=10^9 nmol.

b. 96 well UV plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. In order to ensure the accuracy of the experimental results, it is necessary to take 1-2 samples for pre-experiment. When ΔA is greater than 0.6, it is recommended to dilute the crude enzyme solution with the extraction solution before measuring. When ΔA is less than 0.01, the reaction time (10 min or 15 min) can be extended to measure.
2. The blank tube is a detection hole for detecting the quality of each reagent component. Under normal circumstances, the change does not exceed 0.01.

Experimental example:

1. Take 0.1g geranium and add 1ml extract for homogenization and grinding. After taking the supernatant, operate according to the determination steps. The results show that $\Delta A_T=A1_T-A2_T$= 1.013-0.9753 = 0.0377, $\Delta A_B = A1_B - A2_B$ = 0.8407-0.8392 = 0.0015, $\Delta A = \Delta A_T - \Delta A_B$ = 0.0377-0.0015 = 0.0362.
The activity of PEPC (U/g mass) = 321 × ΔA ÷ W = 321 × 0.0362 ÷ 0.1 = 116.202 U/g mass.
2. Take 0.1g aloe vera and add 1ml extract for homogenization and grinding. After taking the supernatant, operate according to the determination steps. The results are as follows: $\Delta A_T = A1_T - A2_T$ = 0.7049-0.6699 = 0.035, $\Delta A_B = A1_B - A2_B$ = 0.8407-0.8392 = 0.0015, $\Delta A = \Delta A_T - \Delta A_B$ = 0.035-0.0015 = 0.0335
PEPC activity (U/g mass) = 321 × Δ A ÷ W = 321 × 0.0355 ÷ 0.1 = 107.535 U/g mass.

References：

[1] Zhang Y H, Wang Z M, Huang Q, et al. Phosphoenolpyruvate carboxylase activity in ear organs is related to protein concentration in grains of winter wheat[J]. Journal of cereal science, 2008, 47(2): 386-391.

Related Products：

NA0809/NA0567 Hexokinase (HK) Activity Assay Kit
NA0826/NA0584 Pyruvate Kinase (PK) Activity Assay Kit
NA0827/NA0585 Phosphofructokinase (PFK) Activity Assay Kit