

Pyruvate Carboxylase(PC) Activity Assay Kit

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

Operation Equipment: Microplate Reader

Cat No: AK0316

Size:100T/96S

Components:

Extract solution: Liquid 110 mL×2. Storage at 4°C .

Reagent I: Liquid 15 mL×1. Storage at 4°C .

Reagent II: Liquid 5 mL×1. Storage at 4°C .

Reagent III: Powder×1. Storage at -20°C . Dissolve with 3 mL of distilled water, store at -20°C . The prepared reagent can separate into several tubes to avoid repeated freezing and thawing.

Reagent IV: Powder×1. Storage at -20°C . Dissolve with 2 mL of distilled water, store at -20°C . The prepared reagent can separate into several tubes to avoid repeated freezing and thawing.

Reagent V: Liquid 2 mL×1. Storage at 4°C .

Reagent VI: Liquid 5 μL×1. Storage at 4°C .

Reagent VI Diluent Solution: Liquid 5 mL×1. Storage at 4°C .

Product Description:

Pyruvate carboxylase (PC, EC 6.4. 1. 1) is widely present in mitochondria of animals, molds and yeast, but is not found in plants and most bacteria. PC is the main postreaction for oxaloacetate, and is the first rate-limiting enzyme in the gluconeogenesis process.

PC irreversibly catalyzes pyruvate, ATP, CO₂ and water to oxaloacetate, ADP and Pi, malic dehydrogenase further catalyzes the formation of malic acid and NAD⁺ from acetoacetic acid and NADH. The enzyme activity of PC can be reflected by detecting the oxidation rate of NADH at 340 nm.

Reagents and Equipment Required but Not Provided:

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

Procedure:

I. Complex extraction:

- 1) Collecting 0.1 g of tissue or 5 million cells, add 1 mL of Extract solution, grinding on ice with mortar/homogenizer.
- 2) Centrifuge at 1000 ×g for 10 minutes at 4°C ,
- 3) Take the supernatant to other tube and centrifuge at 11000 ×g for 15 minutes at 4°C
- 4) The supernatant is used to detect PC that leaking from mitochondria, which shows the effect of mitochondrial extraction.

5) Add 1 mL of Extract solution to the sediment, splitting with ultrasonic (power 20%, work time 5s, interval 10s, repeat 12 times), used to detect the enzyme activity of PC and protein content.

II. Determination procedure:

- 1) Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
- 2) Preheat Reagent I at 37°C for 15 minutes.
- 3) Diluent Reagent VI according to the volume ratio of Reagent VI: Reagent VI Diluent Solution = 1:428(V:V), prepare the reagent when it will be use.
- 4) Working solution: make the solution as the volume ratio of Reagent II: Reagent III: Reagent IV= 2:1:1, prepare the reagent when it will be used.
- 5) Add the following reagents:

Reagent (μL)	Blank tube (B)	Test tube (T)
Reagent I	90	90
Working solution	64	64
Reagent V	16	16
Reagent VI	20	20
Sample	-	10
Distilled water	10	-

Add the above reagents to the micro quartz cuvette/96 well flat-bottom UV plate in order. Mix thoroughly. Detect the absorbance at 340 nm at the time of 10 seconds record as A_{T1} or A_{B1} . Then place dishes with the reaction solution in a 37°C water bath or incubator for 2 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 130 seconds which record as A_{T2} or A_{B2} . $\Delta A_T = A_{T1} - A_{T2}$, $\Delta A_B = A_{B1} - A_{B2}$, $\Delta A = \Delta A_T - \Delta A_B$. The blank tube only need to test once or twice.

III. Calculation:

A. micro quartz cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every milligram of protein.

$$PC \text{ Activity (U/mg prot)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (V_s \times C_{pr}) \div T = 1607 \times \Delta A \div C_{pr}$$

ϵ : NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d : Light path of cuvette, 1 cm;

V_{rv} : Total reaction volume, 2×10^{-4} L;

V_s : Sample volume (mL), 0.01 mL;

C_{pr} : Sample protein concentration (mg/mL);

T : Reaction time (min), 2 minutes;

10^9 : 1 mol = 10^9 nmol.

B. 96 well flat-bottom UV plate

Change the d - 1 cm in the above formula to d -0.6 cm for calculation.

Note:

1. Take one or two different samples for prediction before test. It is recommended to dilute the crude enzyme solution with the Extract solution before the determination if the $\Delta A > 0.8$. While, extending the response time (5 minutes or 10 minutes) if $\Delta A < 0.01$.
2. The blank tube is a detection hole for detecting the quality of each reagent component, and normally that the change of ΔA_B does not exceed 0.05.
3. The protein concentration of the sample needs to be determined by yourself. Since the Extract solution contains a relatively high protein concentration (about 1 mg/mL), the protein concentration of the Extract solution must be deducted when measuring the protein concentration of the sample.
4. It is recommended to use the sample protein concentration to calculate the enzyme activity. If the sample fresh weight is used to calculate, the enzyme activity of cytoplasmic extract needs to be measured, and the sum of supernatant and precipitation enzyme activity is the total enzyme activity.
5. Reagents in this kit are sufficient to complete 100 tube reactions.
6. Appendix: calculation formula of sample weight: (sample test number is 100T/48S)

A. micro quartz cuvette

1) Supernatant:

Unit definition: One unit of enzyme activity is the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every gram of tissue.

$$\text{PC Activity (U/g weight)} = [\Delta A1 \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \div V_e \times V_s) \div T = 1607 \times \Delta A1 \div W$$

$\Delta A1$: Supernatant absorbance;

ϵ : NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d : Light path of cuvette, 1 cm;

V_{rv} : Total reaction volume, 2×10^{-4} L;

V_s : Sample volume (mL), 0.01 mL;

V_e : Extraction solution, 1 mL;

C_{pr} : Sample protein concentration (mg/mL);

T : Reaction time (min), 2 minutes;

10^9 : 1 mol = 10^9 nmol;

W : Sample weight, g.

2) Sediment:

Unit definition: One unit of enzyme activity is the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every gram of tissue.

$$\text{PC Activity (U/g weight)} = [\Delta A2 \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \div V_e \times V_s) \div T = 1607 \times \Delta A2 \div W$$

$\Delta A2$: Sediment absorbance;

ϵ : NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d : Light path of cuvette, 1 cm;

V_{rv} : Total reaction volume, 2×10^{-4} L;

V_s : Sample volume (mL), 0.01 mL;

V_e : Sediment heavy suspension volume, 1 mL;

C_{pr} : Sample protein concentration (mg/mL);

T : Reaction time (min), 2 minutes;

10^9 : 1 mol= 10^9 nmol;

W : Sample weight, g.

3) Total activity

Total activity is the sum of PC activity in supernatant and sediment.

PC (U/g weight)= $1607 \times \Delta A_1 \div W + 1607 \times \Delta A_2 \div W$.

B. 96 well flat-bottom UV plate

Change the d - 1cm in the above formula to d -0.6cm for calculation.

Experimental example:

1. 1 mL of Extract solution is added to 0.1 g of rabbit heart tissue for homogenization. The supernatant is diluted 100 times with Extract solution, and the precipitation was diluted 4 times. Then, measured by micro quartz plate according to the determination steps, Supernatant: the $\Delta A_T = A_{1T} - A_{2T} = 1.0091 - 0.7487 = 0.2604$, $\Delta A_B = A_{1B} - A_{2B} = 0.9948 - 0.9678 = 0.027$, $\Delta A_1 = \Delta A_T - \Delta A_B = 0.2604 - 0.027 = 0.2334$, Precipitate: $\Delta A_T = A_{1T} - A_{2T} = 1.08 - 0.6157 = 0.4643$, $\Delta A_B = A_{1B} - A_{2B} = 0.9948 - 0.9678 = 0.027$,

$\Delta A_2 = \Delta A_T - \Delta A_B = 0.4643 - 0.027 = 0.4373$

Supernatant: the activity of PC (U/g mass) = $1607 \times \Delta A_1 \div W \times 100$ (dilution ratio) = $1607 \times 0.2334 \div 0.1 \times 100 = 375073.8$ U/g mass;

Precipitation: the enzyme activity of PC (U/g mass) = $1607 \times \Delta A_2 \div W \times 4$ (dilution ratio) = $1607 \times 0.4373 \div 0.1 \times 4 = 28109.64$ U/g mass;

The total enzyme activity of PC (U/g mass) = $1607 \times \Delta A_1 \div W \times 100$ (dilution) + $1607 \times \Delta A_2 \div W$
= $1607 \times 0.2334 \div 0.1 \times 100 + 1607 \times 0.4373 \div 0.1 \times 4 = 403183.44$ U/g mass.

References:

[1] Esmail S. Kakey, Amez A. Ismael. Evaluation of Oxidative Stress Status in Aged Human in relation to some Diseases. International Conference on Pure and Applied Sciences. August 2018;

Related Products:

AK0276/AK0275 Fructose 1,6-bisphosphatase(FBP) Activity Assay Kit

AK0141/AK0140 Glucose-6-phosphatase Activity Assay Kit

AK0143/AK0142 Phosphoenolpyruvate Carboxykinase(PEPCK) Activity Assay Kit