

## Pyruvate Decarboxylase (PDC) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** BC1070

**Size:** 50T/48S

### Components:

**Extract Solution:** Liquid 60 mL×1. Storage at 2-8°C. Contains insoluble substance. Shake well before use. The unused reagent can be stored at 2-8°C for 1 month.

**Reagent IA:** Liquid 28 mL×1. Storage at 2-8°C.

**Reagent IB:** Powder×1. Storage at -20°C.

**Reagent IC:** Liquid 2 mL×1. Storage at 2-8°C.

**Reagent IIA:** Liquid 7 mL×1. Storage at 2-8°C.

**Reagent IIB:** Powder×1. Storage at -20°C. Add 0.6mL distilled water to dissolve the reagent before use, and store the inexhaustible reagent separately at -20°C for 4 weeks, avoid repeated freezing and thawing.

**Reagent IIC:** Powder×1. Storage at -20°C. Add 1ml distilled water to dissolve the reagent before use, and store the inexhaustible reagent separately at -20°C for 4 weeks, avoid repeated freezing and thawing.

**Reagent III:** Liquid 20 mL×1. Storage at 2-8°C.

**Reagent I:** Add reagent IB and reagent IC to reagent IA and dissolve thoroughly before use. Separately store at -20°C for 4 weeks, avoid repeated freezing and thawing.

**Reagent II:** Take 2.625mL of reagent IIA, 0.225mL of reagent IIB and 0.15mL of reagent IIC, mix these reagents well (3mL in total, about 30T) before use.

### Product Description:

Pyruvate Decarboxylase (PDC) exists in yeast mainly, which is one of the key enzymes in ethanol fermentation. PDC catalyzes pyruvate decarboxylation to form acetaldehyde.

Ethanol dehydrogenase (ADH) is added to further catalyze the reduction of acetaldehyde by NADH to produce ethanol and NAD<sup>+</sup>. NADH has a absorbance at 340 nm but NAD<sup>+</sup> not, the activity of PDC can be calculated by measuring decrease rate of absorption at 340 nm.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, Water-Bath/Constant Temperature Incubator, Desk Centrifuge, Adjustable Pipette, 1 mL Quartz Cuvette, Mortar/Homogenizer/Cell ultrasonic Crusher, Ice and Distilled Water.

### Protocol:

#### I. Sample extraction:

1. Bacteria:

Suggested 5 million bacteria/cell with 1 mL of Extract solution. Splitting bacteria and cells

with ultrasonic (ice bath, power 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 16000×g for 20 minutes at 4°C, take the supernatant and place it on ice for test.

2. Tissue:

Add 1 mL of Extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 16000 ×g for 20 minutes at 4°C, take the supernatant and place it on ice for test.

3. Serum:

Detect directly.

**II. Procedure:**

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat Reagent I and Reagent III at 37°C(mammal) or 25°C(other species) in water bath for 30 minutes.
3. Add the following reagents:

| Reagent name (μL) | Test tube (T) | Blank tube (B) |
|-------------------|---------------|----------------|
| Reagent I         | 500           | 500            |
| Reagent III       | 300           | 300            |
| Reagent II        | 100           | 100            |
| Sample            | 100           | -              |
| Distilled water   | -             | 100            |

Mix thoroughly, detect the absorbance at 340 nm at 10s and 70s,  $\Delta A(\text{Test}) = \Delta A(T) = A1(10s) - A2(70s)$ ,  $\Delta A(\text{Blank}) = \Delta A(B) = A3(10s) - A4(70s)$ .  $\Delta A = (A1 - A2) - (A3 - A4)$ . Blank tube only needs to do 1-2 times

**III. Calculation:**

**1. Serum:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37°C(mammal) or 25°C(other species) every milliliter of serum.

$$PDC(U/mL) = \Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6 \div V_s \div T = 1.6 \times \Delta A$$

**2. Tissue:**

**1) Protein concentration:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37°C(mammal) or 25°C(other species) every milligram of tissue protein.

$$PDC(U/mg \text{ prot}) = \Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6 \div (V_s \times C_{pr}) \div T = 1.6 \times \Delta A \div C_{pr}$$

**2) Sample weight:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37°C(mammal) or 25°C(other species) every gram of tissue.

$$PDC(U/g) = \Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6 \div (W \div V_e \times V_s) \div T = 1.6 \times \Delta A \div W$$

### 3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 $\mu$ mol NADH per minute at 37°C(mammal) or 25°C(other species) every 10 thousand bacteria or cells.

$$PDC (U/10^4 \text{ cell}) = \Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6 \div (V_s \div V_e \times 500) \div T = 3.2 \times 10^{-3} \times \Delta A \div C_{pr}$$

$\epsilon$ : NADH molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{rv}$ : Total reaction volume, 1 mL=0.001 L;

$V_s$ : Extract solution volume (mL), 0.1 mL;

$C_{pr}$ : Sample protein concentration (mg/mL); need to detect separately;

T: Reaction time (min), 1 minute;

W: Sample weight(g);

$V_e$ : Extract solution volume, 1 mL;

500: amount of cell or bacteria, 5 million.

$10^6$ : 1mol=10<sup>6</sup> $\mu$ mol

#### Note:

1. During the experiment, the Reagent II and sample were placed on ice to avoid denaturation and inactivation.
2. Keep the reaction solution in the cuvette at 37°C or 25°C. Take a small beaker and fill it with a certain amount of distilled water at 37°C or 25°C. Then put the beaker in a water bath at 37°C or 25°C. In the course of the reaction, the cuvette and the reaction liquid are put in this beaker.
3. It is better to do the experiment with two people at the same time, one person for colorimetric and the other for timing to ensure the accuracy of the experiment results.
4. React time can be extended if the change value in one minute is low, note to modify the calculation formula.

#### Experimental instances:

1. Take 0.1g of Scindapsus leaf, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate  $\Delta A = (A_1 - A_2) - (A_3 - A_4) = (0.945 - 0.93) - (0.716 - 0.714) = 0.013$ , calculate the enzyme activity according to sample weight:

$$PDC (U/g) = 1.6 \times \Delta A \div W = 1.6 \times 0.013 \div 0.1 = 0.208 \text{ U/g.}$$

2. Take 0.1g of mouse liver, add 1mL of extract solution, homogenate and grind. Take the supernatant, 40 times dilution, according to the measured steps, measure and calculate  $\Delta A = (A_1 - A_2) - (A_3 - A_4) = (0.602 - 0.322) - (0.716 - 0.714) = 0.278$ , calculate the enzyme activity according to sample weight:

$$PDC (U/g \text{ weight}) = 1.6 \times \Delta A \div W = 1.6 \times 0.278 \div 0.1 \times 40 (\text{Dilution Ratio}) = 177.92 \text{ U/g weight.}$$

**References:**

Chong Li, Shi Gao, Xiaotong Li, et al. Efficient metabolic evolution of engineered *Yarrowia lipolytica* for succinic acid production using a glucose-based medium in an in situ fibrous bioreactor under low-pH condition. *Biotechnology for Biofuels*. August 2018;(IF5.452)

**Related products:**

|               |                                    |
|---------------|------------------------------------|
| BC0590/BC0595 | Free fatty Acids(FFA) Assay Kit    |
| BC2340/BC2345 | Lipase(LPS) Activity Assay Kit     |
| BC0320/BC0325 | Plant Lipoxygenase (LOX) Assay Kit |
| BC0750/BC0755 | Aldehyde Dehydrogenase (ALDH)      |