Pyruvate Decarboxylase (PDC) Activity Assay Kit

Catalog No.: BC00067

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Pyruvate Decarboxylase (PDC) Activity Assay Kit		
Detection Methods	Colorimetric		
Sample type	Serum, plasma, animal and plant tissues, cells		
Detection Type	Enzyme activity		
Detection instrument and wavelength	Microplate reader (340 nm)		
Range	0.67-27.73 U/L		
Sensitivity	0.67U/L		

Product Introduction

Pyruvate decarboxylase (PDC) mainly exists in yeast and is one of the key enzymes in ethanol fermentation.

Detection principle

PDC catalyzes the decarboxylation of pyruvate to produce acetaldehyde. Acetaldehyde reacts under the action of alcohol dehydrogenase (ADH) and catalyzes the conversion of NADH into NAD+. NADH has a characteristic absorption peak at 340nm. The PDC activity can be calculated by measuring the rate of decrease of light absorption at 340nm.

PDC ADH

Pyruvate _______ acetaldehyde + NADH <_______ ethanol + NAD +

When this kit detects tissue samples and cell samples, the total protein concentration needs to be determined. It is recommended to use the BCA kit for determination. (Cat. No.: BC00006)

Product Composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Buffer	30mL	-20°C, store at 2-8°C after use.
Reagent 2	Substrate A	1 bottle	-20°C, store at 2-8°C away from light after
Reagent 3	Enzyme	1 piece	Store at 2-8°C before use and -20°C after
Reagent 4	Substrate B	1 piece	Store at 2-8°C before use and -20°C away

Consumables 1	96 -well	1 plate	RT
Consumables 2	96-well	2 pieces	RT

Storage Conditions

After receiving the test kit, please store it according to "Product Components-Storage Method". The validity period is 6 months.

Preparation before the experiment

Sample processing

- 1. Tissue sample homogenization: Tissue samples were homogenized with physiological saline (0.9% NaCl), and then centrifuged at 12,000 × g for 10 min at 4°C. After centrifugation, the supernatant was collected for testing. A portion of the supernatant was retained for protein concentration determination.
- 2. Serum (plasma) samples: direct detection.

· Preparation of the kit

- 1. Before testing, the reagents in the kit were equilibrated to room temperature.
- 2. Preparation of Reagent 2 working solution: Add 5 mL of double distilled water to one bottle of Reagent 2, mix well, and place on an ice box away from light for use. The unused portion can be stored at -20°C away from light for 7 days to avoid repeated freezing and thawing.
- 3. reagent 3 working solution: Take one vial of reagent 3 and add 100 μL of double distilled water, mix well, place on an ice box away from light for later use, and store the unused portion at -20°C for 3 days to avoid repeated freezing and thawing.
- reagent IV working solution: Take one vial of reagent IV and add 2 mL of double distilled water, mix well, place on an ice box away from light for later use, and store the unused portion at -20°C for 3 days to avoid repeated freezing and thawing.

Operation process

- 1. 160 μL of reagent 1 to the blank well and 120 μL of reagent 1 to the assay well .
- 2. Add 20 µL of reagent 2 working solution to each well in step (1).
- 3. Add 37 µL of double distilled water to the measurement well in step (2).
- 4. IV working solution to the assay well in step (3).
- 5. 3 working solution to the assay well in step (4).
- 6. Add 20 µL of the sample to be tested to each well in step (1).
- 7. value of the blank well and the test well at 1min and 3min A_2 was measured by the microplate reader at a wavelength of 340nm A_1 , and the change in OD value ΔA was calculated, $\Delta A = A_1 A_2$

The operation table is as follows:

	Assay well (μl)	Blank well (μl)
Reagent 1	120	160
Reagent 2	20	20
Double distilled water	37	-
Reagent 4	2	-
Reagent 3	1	-
Samples to be tested	20	20

value of the blank well and the test well at 1min and 3min A_2 was measured by the microplate reader at a wavelength of 340nm A_1 , and the change in OD value ΔA was calculated, $\Delta A = A_1 - A_2$

Result calculation

The calculation formula of pyruvate decarboxylase (PDC) activity in tissue or cell samples is:

PDC activity (U/ gprot) =
$$\frac{\Delta A test - \Delta A b lank}{\epsilon \times d}$$
 : Cpr ÷ T × f × 10⁶

the amount of enzyme required to catalyze NADH to produce 1 µmol NAD per minute per gram of tissue or cell protein is one unit of activity.

The calculation formula of pyruvate decarboxylase (PDC) activity in serum and plasma samples is:

PDC activity (U/L) =
$$\frac{\Delta A test - \Delta A b lank}{\epsilon \times d}$$
 \div T × f × 10⁶

the amount of enzyme required to catalyze NADH to produce 1µmol NAD per minute per liter of serum/plasma is one activity unit.

Note: $\triangle A$ measurement: Determine the change in OD value of the well $A_1.A_2$

 ΔA blank: blank well change OD value A_1 - A_2

ε: NADH molar extinction coefficient, 6.22× 10³L/(mol•cm)

d: ELISA plate light path, 0.6 cm

Cpr: protein concentration of the sample to be tested, gprot /L

f: dilution factor of the sample before adding it to the detection system

T: reaction time, 2min

 10^6 : 1mol = $10^6 \mu$ mol

Notes

1. During the sample addition process, pay attention to mixing evenly to avoid affecting the test

results.

- 2. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
- 3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
- 4. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.