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CheKine™ Micro Pyruvate Decarboxylase (PDC) Assay Kit

Cat #: KTB1125 **Size:** 48 T/96 T

	Micro Pyruvate Decarboxylase (PDC) Assay Kit		
REF	**Cat #:** KTB1125	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pyruvate decarboxylase (PDC) is mainly found in yeast and is one of the key enzymes in ethanol fermentation. It catalyzes the decarboxylation of pyruvate to acetaldehyde. CheKine™ Micro Pyruvate Decarboxylase (PDC) Activity Assay Kit provides a simple method for detecting PDC activity in animal and plant tissues, cells, bacteria, serum, plasma. The principle is that PDC catalyzes the decarboxylation of pyruvate to produce acetaldehyde, and alcohol dehydrogenase (ADH) is added to further catalyze the reduction of acetaldehyde by NADH to produce ethanol and NAD^+. NADH has an absorption peak at 340 nm, but NAD^+ does not. PDC activity is calculated by measuring the rate of decrease in light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	9 mL	18 mL	-20℃, protected from light
Reagent Ⅱ	10 mL	20 mL	4℃
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	15 μL	30 μL	-20℃, protected from light
Reagent Ⅴ	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent: Before use, dissolve Reagent Ⅲ and Reagent Ⅳ with some Reagent Ⅱ, and then transfer them all to Reagent Ⅱ for use. The remaining reagents should be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅴ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma and serum: Direct detection.

It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheated Reagent Ⅰ in a 25℃ water bath for 30 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Blank Well (μL)	Test Well (μL)
Reagent Ⅰ	140	140
Working Reagent	20	20
Reagent Ⅴ	20	20
Sample	0	20
Deionized Water	20	0

4. After mixing quickly, record the absorbance values of 10 s and 70 s at 340 nm with a microplate reader. The test well is marked as A_1 and A_2, and the blank well is marked as A_3 and A_4. Finally calculate $\Delta A_{Test}=(A_1-A_2)-(A_3-A_4)$.

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculation of PDC activity in serum (plasma)

Unit definition: an enzyme activity unit defines as each mL of serum (plasma) catalyzes the oxidation of 1 nmol NADH per min in

the reaction system at 25℃.

PDC (U/mL)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T=**3,215×ΔA_{Test}**

2. Calculation of PDC activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: an enzyme activity unit defines as 1 mg of tissue protein catalyzes the oxidation of 1 nmol NADH per min in the reaction system at 25℃.

PDC (U/mg prot)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T=**3,215×ΔA_{Test}÷Cpr**

(2) Calculation according to the weight of the sample

Unit definition: an enzyme activity unit defines as 1 g tissue catalyzes the oxidation of 1 nmol NADH per min in the reaction system at 25℃.

PDC (U/g fresh weight)=[ΔA_{Test}×V_{Total} ÷(ε×d)×10^9]÷(W÷$V_{Extraction}$×V_{Sample})÷T=**3,215×ΔA_{Test}÷W**

3. Calculation of PDC activity in bacteria or cells

Calculation by the number of bacteria or cells

Unit definition: an enzyme activity unit defines as 10,000 bacteria or cells catalyze the oxidation of 1 nmol NADH per min in the reaction system at 25℃.

PDC (U/10^4)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Extraction}$×500)÷T=**6.4×ΔA_{Test}**

Where: V_{Total}: the total volume of the reaction system, 0.2mL=2×10^{-4} L, $V_{Extraction\ Buffer}$: the volume of the Extraction Buffer, 1 mL; V_{Sample}: the volume of the supernatant in the reaction system, 0.02 mL; ε: NADH molar extinction coefficient , 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration, mg/mL; T: reaction time, 1 min; W: sample weight, g; 500: total number of bacteria or cells , 5x10^6; 10^9: unit conversion factor, 1 mol=10^9 nmol.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Precautions

1. Extraction Buffer contains insoluble substance, shake well before use.
2. If the change value of 1 min is small, the reaction time can be prolonged and the calculation formula time can be modified.

Typical Data

Take 0.1 g of plant leaves and add 1 mL Extraction Buffer for homogenization and grinding, take supernatant, then follow the determination steps, and measure with 96-well UV plate:

A_1=1.263, A_2=1.236, A_3=0.237, A_4=0.236, ΔA_{Test}=(A_1-A_2)-(A_3-A_4)=(1.263-1.236)-(0.237-0.236)=0.026. Calculate the PDC activity according to the weight of the sample: PDC (U/g fresh weight)=3,215×ΔA_{Test}÷W=3,215×0.026÷0.1=835.9 U/g weight.

Recommended Products

Catalog No.	Product Name
KTB1020	CheKine™ Micro Coenzyme Ⅰ NAD(H) Assay Kit
KTB1021	CheKine™ Micro NADH Oxidase (NOX) Assay Kit
KTB1022	CheKine™ Micro NAD+ Kinase (NADK) Activity Assay Kit
KTB1010	CheKine™ Micro Coenzyme Ⅱ NADP(H) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.