PRODUCT INFORMATION & MANUAL

Pyruvate Decarboxylase/PDC Activity Assay Kit (Colorimetric)

NBP3-25782

For research use only.

Not for diagnostic or therapeutic procedures.

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Pyruvate Decarboxylase/PDC Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25782

Method: Colorimetric method

Specification: 96T (Can detect 94 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.67 U/L

Detection range: 0.67-27.73 U/L

Average intra-assay CV (%): 2.8

Average inter-assay CV (%): 3.1

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure pyruvate decarboxylase (PDC) activity in serum, plasma, tissue and cell samples.

▲Detection principle

Pyruvate decarboxylase (PDC) mainly exists in yeast and is one of the key enzymes in ethanol fermentation. PDC catalyzes pyruvate decarboxylate to acetaldehyde, which reacts under the action of ethanol dehydrogenase (ADH), and catalyzes NADH to convert into NAD^+. NADH has a characteristic absorption peak at 340 nm. The PDC activity can be calculated by measuring the OD value at 340 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 2	Substrate A	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Substrate B	Powder × 2 vials	-20℃ , 12 months, shading light
	UV Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (330-350nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. All reagents are stored strictly with shading light and avoid from repeated freezing and thawing.
2. The change of OD per unit time (min) should be controlled within 0.2.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:

   Dissolve a vial of reagent 2 with 5 mL of double distilled water. Preserve it on the ice box with shading light for use and the prepared solution can be stored at -20℃ with shading light for 3 days.
3. Preparation of reagent 3 working solution:

   Dissolve a vial of reagent 3 with 1.2 mL of double distilled water. Preserve it on the ice box with shading light for use and the prepared solution can be stored at -20℃ with shading light for 7 days.
4. Preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 with 1.2 mL of double distilled water. Preserve it on the ice box with shading light for use and the prepared solution can be stored at -20℃ with shading light for 3 days.

▲Sample preparation

1. Serum and plasma samples:

   Detect the sample directly.

2. Tissue sample:

   Weigh the tissue accurately and add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 12000 g for 10 min at 4℃ , then take the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Add normal saline (0.9% NaCl) at a ratio of cell number (10^6): 0.9% NaCl (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 12000 g for 10 min at 4℃ , then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.67-27.73 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	25-50
10% Mouse kidney tissue homogenate	25-50
10% Mouse heart tissue homogenate	25-50
Rat serum	1
Rabbit plasma	1
HL-60 cell	3-5

Note:The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	S1	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S2	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S3	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S4	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S5	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S6	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
H	S7	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

Note: A, blank wells; S1-S94, sample wells.

▲Detailed operation steps

1) Blank well: Add 20 μL of double distilled water to the wells.

   Sample well: Add 20 μL of sample to the wells.

2) Add 120 μL of reagent 1 into each well.

3) Add 20 μL of reagent 3 working solution into each well.

4) Add 20 μL of reagent 4 working solution into each well.

5) Add 20 μL of reagent 2 working solution into each well.

6) Mix fully, measure the OD value of each well at 1 min and 3 min respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\Delta A = A_1 - A_2$.

▲Summary operation table

	Blank well	Sample well
Double distilled water (μL)	20	
Sample (μL)		20
Reagent 1 (μL)	120	120
Reagent 3 working solution (μL)	20	20
Reagent 4 working solution (μL)	20	20
Reagent 2 working solution (μL)	20	20
Measure the OD value of each well at 1 min and 3 min respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\Delta A=A_1-A_2$.		

▲Calculation

1. Tissue and Cell:

Definition: The amount of PDC in 1 g tissue or cell protein per 1 minute that hydrolyze the NADH to produce 1 μmol NAD at 37℃ is defined as 1 unit.

$$\text{PDC activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \div C_{pr} \div T \times f \times 10^6$$

2. Serum/Plasma:

Definition: The amount of PDC in 1 L liquid sample per 1 minute that hydrolyze the NADH to produce 1 μmol NAD at 37℃ is defined as 1 unit.

$$\text{PDC activity (U/L)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \div T \times f \times 10^6$$

Note:

ΔA_{Sample}: The change OD value of sample well, A_1-A_2.

ΔA_{Blank}: The change OD value of blank well, A_1-A_2.

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/(mol•cm).

d: The optical path of microplate, 0.6 cm.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

T: The reaction time, 2 min.

10^6: 1 mol = 10^6 μmol.

Appendix I Data

▲Example analysis

For 10% mouse liver tissue homogenate, dilute for 50 times, and carry the assay according to the operation table. The results are as follows:

The A_1 of the blank well is 1.054, the A_2 of the blank well is 1.053, the A_1 of the sample well is 0.674, the A_2 of the sample well is 0.448, the concentration of protein in sample is 14.18 gprot/L, and the calculation result is:

PDC activity (U/gprot) = ((0.674 - 0.448) - (1.054 - 1.053)) ÷ (6220 × 0.6) ÷ 14.18 ÷ 2 × 50 × 10^6 = 106.29 U/gprot