

PRODUCT INFORMATION & MANUAL

Pyruvate Dehydrogenase/PDH Activity Assay Kit (Colorimetric) NBP3-25783

For research use only.

Not for diagnostic or therapeutic procedures.

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Pyruvate Dehydrogenase/PDH Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25783

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.28 U /L

Detection range: 0.28-168.91 U/L

Average intra-assay CV (%): 2.0

Average inter-assay CV (%): 10.0

Average recovery rate (%): 95

- ▲ 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 measure pyruvate dehydrogenase (PDH) activity in serum, plasma, urine, animal tissue and cell samples.

▲ Detection principle

Pyruvate dehydrogenase (PDH) catalyzed the oxidative decarylation of pyruvate into acetyl CoA, which plays an important role in the metabolism of mitochondrial respiratory chain and is associated with the aging of cancer genes. Pyruvate can be decarboxylated by PDH under the action of CoA. Meanwhile, NAD⁺ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of PDH can be calculated by measuring the change of absorbance value at 450 nm.

▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20 , 12 months
Reagent 2	Buffer Solution	40 mL × 1 vial	-20 , 12 months, shading light
Reagent 3	Substrate A	powder × 2 vials	-20 , 12 months, shading light
Reagent 4	Substrate B	powder × 2 vials	-20 , 12 months, shading light
Reagent 5	Chromogenic Agent	6 mL×1 vial	-20 , 12 months, shading light
Reagent 6	Standard	powder × 2 vials	-20 , 12 months, shading light
	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



1 Instruments

Centrifuge, Microplate reader (440-450 nm, optimum wavelength: 450 nm)

▲ 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.

Pre-assay preparation

▲ Reagent preparation

- 1. Bring all reagents to room temperature before use.
- 2. Preparation of reagent 3 working solution:

Dissolve a vial of reagent 3 with 0.5 mL double distilled water and mix fully. Preserve it on ice box with shading light for use The prepared solution can be stored at -20 with shading light for 3 days, avoiding repeated freezing-thawing.

3. Preparation of reagent 2 working solution:

Mix the reagent 2 and reagent 3 working solution at the ratio of 100:1 fully. Prepare the fresh needed amount before use. The prepared solution should be used up on the same day.

4. Preparation of reagent 4 working solution:

Dissolve a vial of reagent 4 with 1.6 mL double distilled water and mix fully. Preserve it on ice box with shading light for use The prepared solution can be stored at -20 with shading light for 7 days.

5. Preparation of 0.5 mmol/L standard solution:

Dissolve a vial of reagent 6 with 1 mL double distilled water and mix fully. Preserve it on ice box with shading light for use The prepared solution can be stored at -20 with shading light for 7 days.

▲ Sample preparation

1. Serum (plasma) and other liquid samples:

Detect directly. (if the sample is turbidity, centrifugation at 12000 g for 10 min before use).

2. Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for $1\sim2$ times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (1×10^6): reagent 1 (μ L)=1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant 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 (0.28-168.91 U/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	2-3
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse liver tissue homogenate	2-3
10% Mouse kidney tissue homogenate	1
10% Mouse lung tissue homogenate	1
1×10^6 HL-60 cells	1
Rat serum	1
Rat plasma	1
Human urine	1

Note: The diluent is reagent 1.

Assay protocol

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
А	А	А	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
В	В	В	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
С	С	С	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	Е	Е	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
Н	Н	Н	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲ Detailed operation steps

1. The preparation of standard curve

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.10, 0.20, 0.25, 0.30, 0.40, 0.50 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	0.5 mmol/L standard solution (μL)	
А	0	0	200
В	0.05	20	180
С	0.10	40	160
D	0.20	80	120
Е	0.25	100	100
F	0.30	120	80
G	0.40	160	40
Н	0.50	200	0

2. The measurement of samples

(1) Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Control well: Add 20 µL of sample to the corresponding wells.

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

- (2) Add 140 µL of reagent 2 working solution to each well.
- (3) Add 20 μL of reagent 4 working solution to standard well and sample well. Add 20 μL of double distilled water to control well.
- (4) Add 20 μL of reagent 5 to each well.
- (5) Measure the OD value of each well at 20 s and 3 min 20 s respectively at 450 nm with microplate reader, recorded as A_1 , A_2 , $\Delta A = A_1 A_2$. Plot the standard curve with A_2 values.

▲ Summary operation table

Standard well	Control well	Sample well
20		
	20	20
140	140	140
20		20
	20	
20	20	20
	20 140 20	20 140 140 20 20

Measure the OD value of each well at 20 s and 3 min 20 s respectively, recorded as A_1 , A_2 , $\Delta A = A_1 - A_2$. Plot the standard curve with A_2 values.

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y = ax + b.

1. Tissue and cell sample:

Definition: The amount of PDH in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 µmol product at 37 is defined as 1 unit.

=
$$(\Delta A_{\text{sample}} - \Delta A_{\text{control}} - b) \div a \div C_{pr} \div T \times f \times 1000$$

2. Serum, plasma and other fluid sample:

Definition: The amount of PDH in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 µmol product at 37°C is defined as 1 unit.

=
$$(\Delta A_{sample} - \Delta A_{control} - b) \div a \div T \times f \times 1000$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0, the standard is calculated using only A_2 values).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

 $\Delta A_{\text{control}}\!\!:$ The change OD value of control well, A_2 - $A_1.$

T: The time of reaction, 3 min.

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

f: Dilution factor of sample before test.

1000: 1 mmol = 1000 μ mol.

Appendix I Data

▲ Example analysis

For 10% rat kidney tissue homogenate, carry the assay according to the operation table. The results are as follows:

standard curve: y = 1.8769 x - 0.0042, the A_1 of the control well is 0.175, the A_2 of the control well is 0.177, $\Delta A_{\text{Control}} = 0.177 - 0.175 = 0.002$. The A_1 of the sample well is 0.217. the A_2 of the sample well is 0.602, $\Delta A_{\text{Sample}} = 0.602 - 0.217 = 0.385$, the concentration of protein in sample is 10.08 gprot/L, and the calculation result is: PDH activity (U/gprot) = $(0.385 - 0.002 + 0.0042) \div 1.8769 \div 10.08 \div 3 \times 1000 = 6.822$ U/gprot