PRODUCT INFORMATION & MANUAL

Aldehyde Dehydrogenase Activity Assay Kit (Fluorometric)

NBP3-25859

For research use only.
Not for diagnostic or therapeutic procedures.

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Aldehyde Dehydrogenase Activity Assay Kit (Fluorometric)

Catalog No: NBP3-25859

Method: Fluorimetric method

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

Instrument: Fluorimetric Microplate Reader

Sensitivity(According to produced NADH concentration): 0.06 µmol/L

Detection range(According to produced NADH concentration): 0.06–3 µmol/L

Average intra-assay CV (%): 4

Average inter-assay CV (%): 8.4

Average recovery rate (%): 100

- ▲ 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 aldehyde dehydrogenase (ALDH) activity in serum (plasma), animal tissue and cell samples.

▲Detection principle

The main pathway of alcohol metabolism is oxidation of alcohol dehydrogenase (ADH) to acetaldehyde, and then NADH-dependent acetaldehyde dehydrogenase (ALDH) oxidizes to acetic acid.

The detection principle of this kit is that the substrate under the action of aldehyde dehydrogenase transforms NAD^+ into NADH, NADH reacts with the fluorescent probe to form fluorescent substance under the action of enzymes. The activity of ALDH can be calculated by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20℃ , 12 months
Reagent 2	Coenzyme	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Substrate	0.3 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	0.2 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 5	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 6	Standard	Powder × 2 vials	-20℃ , 12 months, shading light
	Black 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

Micropipettor, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 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.

▲ The key points of the assay

Avoid bubbles when adding reaction working solution.

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 1 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 2 days with shading light.
3. Preparation of reaction working solution:

   Mix the reagent 1, reagent 2 working solution and reagent 3 at a ratio of 177:15:0.8 fully. Prepare the fresh needed amount before use and the prepared solution should be used within 6 h.
4. Preparation of reagent 5 working solution:

   Dissolve a vial of reagent 5 with 100 μL of double distilled water, mix fully and preserve it on ice for detection. The prepared solution can be stored at 2-8°C for 1 day with shading light.
5. Preparation of chromogenic working solution:

   Mix the double distilled water, reagent 4 and reagent 5 working solution at a ratio of 280:16:5 fully and preserve it on ice with shading light for detection. Prepare the fresh needed amount before use and the prepared solution should be used within 6 h.
6. Preparation of 300 μmol/L standard:

   Dissolve a vial of reagent 6 with 1.6 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 2 days.

   Preparation of 3 μmol/L standard:

   Dilute 300 μmol/L standard with double distilled water at a ratio of 1:99 fully. Prepare the fresh needed amount before use.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) 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 1~2 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 (10^6): normal saline (0.9% NaCl) (μL) =1: 200. Sonicate or mechanical homogenate 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 (0.06–3 μmol/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	150-160
10% Mouse kidney tissue homogenate	90-150
10% Mouse liver tissue homogenate	150-180
10% Mouse lung tissue homogenate	60-90
10% Mouse brain tissue homogenate	30-40
10% Mouse spleen tissue homogenate	60-150
Mouse plasma	3-5
Jurkat cell	8-12

Note: The diluent is normal saline (0.9% NaCl) or double distilled water.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1’	S9	S9’	S17	S17’	S25	S25’	S33	S33’
B	B	B	S2	S2’	S10	S10’	S18	S18’	S26	S26’	S34	S34’
C	C	C	S3	S3’	S11	S11’	S19	S19’	S27	S27’	S35	S35’
D	D	D	S4	S4’	S12	S12’	S20	S20’	S28	S28’	S36	S36’
E	E	E	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’
H	H	H	S8	S8’	S16	S16’	S24	S24’	S32	S32’	S40	S40’

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

▲Detailed operation steps

2. The preparation of standard curve

Dilute 3 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.6, 0.9, 1.2, 1.8, 2.4, 2.7, 3 μmo/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	3 μmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.6	40	160
C	0.9	60	140
D	1.2	80	120
E	1.8	120	80
F	2.4	160	40
G	2.7	180	20
H	3	200	0

2. The measurement of samples

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

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

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

(2) Add 140 μL of reaction working solution into the standard and sample wells.

Add 140 μL of reagent 1 into the control wells.

(3) Add 20 μL of chromogenic working solution into each wells.

(4) Mix fully with microplate reader for 3 s and react at room temperature with shading light for 5 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Operation table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	20		
Sample (μL)		20	20
Reaction working solution (μL)	140	140	
Reagent 1 (μL)			140
Chromogenic working solution (μL)	20	20	20
Mix fully and incubate at room temperature for 5 min. Measure the fluorescence intensity of each well.			

▲Calculation

Plot the standard curve by using fluorescence value (F) 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 F value of sample. The standard curve is: y= ax + b.

1. For tissue and cell:

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

$$\text{ALDH activity (U/gprot)} = (F_{Sample} - F_{Control} - b) \div a \div T \div C_{pr} \times f$$

2. Serum/plasma sample:

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

Note:

y: $F_{Standard} - F_{Blank}$. (F_{Blank} is the fluorescence value when the standard concentration is 0).

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

F_{Sample}: The fluorescence intensity of sample well.

$F_{Control}$: The fluorescence intensity of control well

T: The time of incubation reaction, 45 min.

C_{pr}: The concentration of protein in tissue or cell , gprot/L.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For mouse liver tissue, take 40 μL of 10% mouse liver tissue homogenate, diluted for 180 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 952 x + 69.8, the average fluorescence value of the control is 413, the average fluorescence value of the sample is 1537, the concentration of protein in sample is 3.51 gprot/L, and the calculation result is:

ALDH activity (U/gprot)=(1537 - 413 - 69.8) ÷ 952 ÷ 5 ÷ 3.51 × 180 = 11.36 U/gprot