（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F044

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.06—3 μmol/L

Elabscience® Aldehyde Dehydrogenase（ALDH）Fluorometric Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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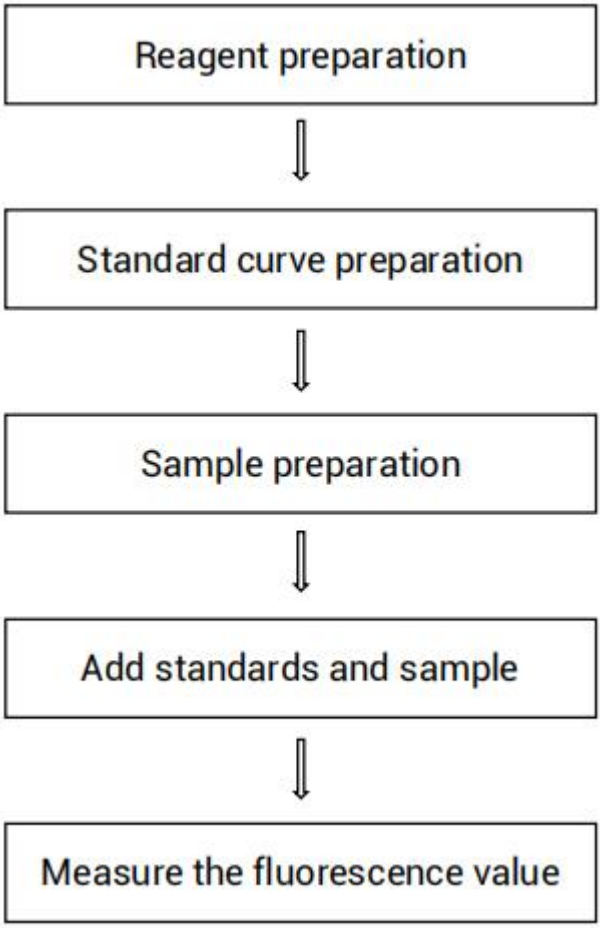
Assay summary



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	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Coenzyme	Powder × 2 vials	-20°C, 12 months shading light
Reagent 3	Substrate	0.3 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months shading light
Reagent 6	Standard	Powder × 2 vials	-20°C, 12 months shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of coenzyme working solution:
Dissolve one vial of coenzyme with 1 mL of double distilled water, mix well. Store at 2-8°C for 2 days protected from light.

③ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 1928 μL of reaction working solution (mix well 1770 μL of buffer solution, 150 μL of coenzyme working solution and 8 μL of substrate). The reaction working solution should be prepared on spot, and used up within 6 hours.

④ The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 100 μL of double distilled water, mix well and keep it on ice for use. Store at 2-8°C for 1 day protected from light.

⑤ The preparation of chromogenic working solution:
Before testing, please prepare sufficient chromogenic working solution

according to the test wells. For example, prepare 301 μL of chromogenic working solution (mix well 280 μL of double distilled water, 16 μL of chromogenic agent and 5 μL of enzyme working solution). The chromogenic working solution should be prepared on spot and used up within 6 hours.

⑥ The preparation of 300 μmol/L standard:

Dissolve one vial of standard with 1.6 mL of double distilled water, Mix well to dissolve. Store at 4°C for 2 days protected from light.

⑦ The preparation of 3 μmol/L standard:

Dilute 10 μL of 300 μmol/L standard with 990 μL of double distilled water and mix well. The 3 μmol/L standard should be prepared on spot.

⑧ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 3 μmol/L standard 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.6**	**0.9**	**1.2**	**1.8**	**2.4**	**2.7**	**3**
3 μmo/L standard (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Avoid bubbles when adding reaction working solution.

Operating steps

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 buffer solution 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue and cell samples:

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.

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

[Note]

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

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

T: The time of incubation reaction, 5 min.

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

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.10	1.50	2.30
%CV	4.2	3.8	4.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.10	1.50	2.30
%CV	8.5	7.9	8.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	0.8	1.5	2.5
Observed Conc. (μmol/L)	0.8	1.5	2.5
Recovery rate (%)	101	99	100

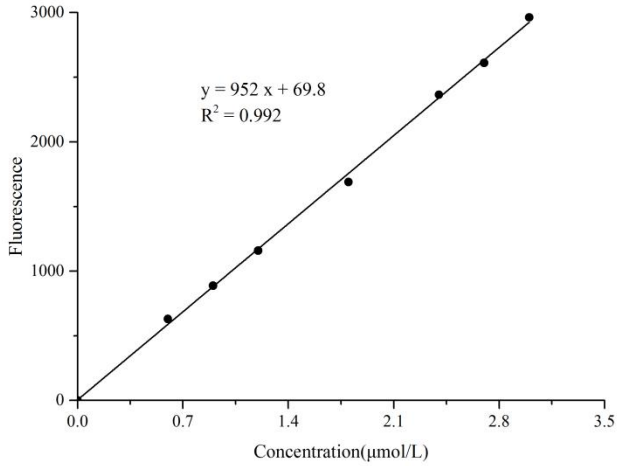
Sensitivity

The analytical sensitivity of the assay is 0.06 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	**0**	**0.6**	**0.9**	**1.2**	**1.8**	**2.4**	**2.7**	**3**
Fluorescence value	361	997	1225	1506	2025	2756	2963	3309
	345	968	1256	1517	2059	2680	2963	3322
Average fluorescence value	353	983	1241	1512	2042	2718	2963	3316
Absoluted fluorescence value	0	630	888	1159	1689	2365	2610	2963



Appendix Π Example Analysis

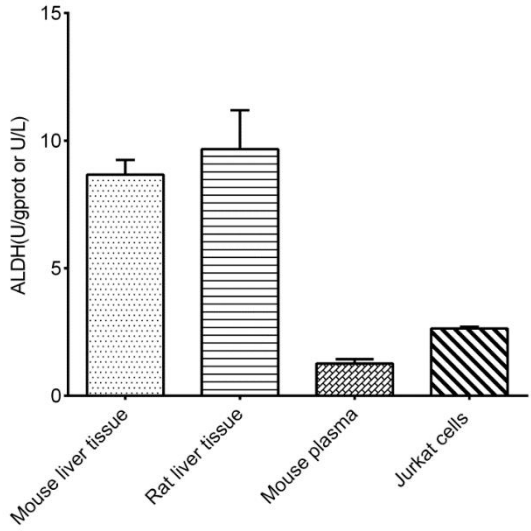
Example analysis：

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 steps. 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

Detect 10% mouse liver tissue homogenate (the concentration of protein is 3.51 gprot/L, dilute for 180 times), 10% rat liver tissue homogenate (the concentration of protein is 11.95 gprot/L, dilute for 180 times), mouse plasma(dilute for 3 times) and Jurkat cell (the concentration of protein is 0.3 gprot/L, dilute for 10 times) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.