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

Catalog No：E-BC-K565-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.01–10.0 U/L

Elabscience® Aldehyde Dehydrogenase (ALDH) 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
Tell: 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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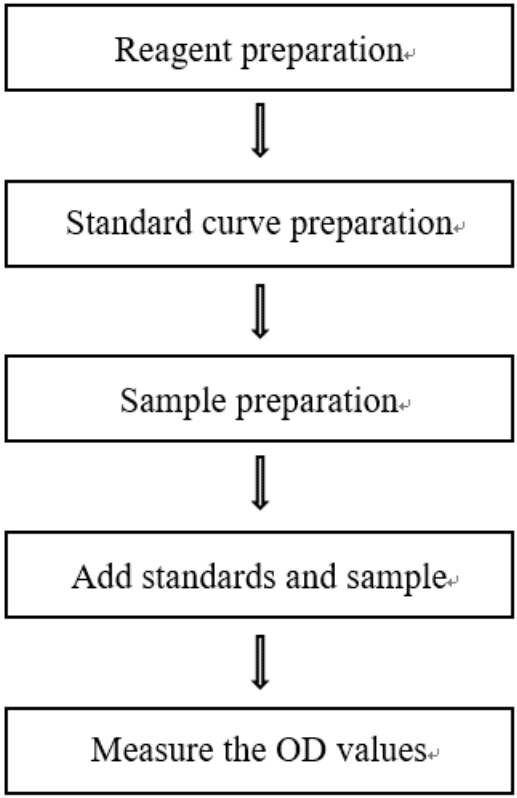
Assay summary



Intended use

This kit can be used to measure aldehyde dehydrogenase (ALDH) activity in serum (plasma) and animal tissue 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, which under the action of electron coupling agent, transfer electrons to WST-8 to produce the yellow product. The activity of ADH can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	9 mL ×1 vial	18 mL ×1 vial	-20℃, 12 months
Reagent 2	Coenzyme	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months shading light
Reagent 3	Substrate	0.08 mL ×1 vial	0.08 mL ×1 vial	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	1.2 mL×1 vial	1.2 mL×2 vials	-20℃, 12 months shading light
Reagent 5	Standard	Powder ×1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Incubator, Centrifuge, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of coenzyme stock solution:
Dissolve a vial of coenzyme with 1 mL double distilled water. Store at 2-8 ℃ for 3 days protected from light.

③ The preparation of coenzyme working solution:
Before testing, please prepare sufficient coenzyme working solution according to the test wells. For example, prepare 600 μL of coenzyme working solution (mix well 60 μL of coenzyme stock solution and 540 μL of buffer solution). The coenzyme working solution should be prepared on spot and use up within 1 h.

④ The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 300 μL of substrate working solution (mix well 5 μL of substrate and 295 μL of double distilled water). Store at 2-8 ℃ for 3 days protected from light.

⑤ The preparation of reaction working solution:
For each well, prepare 160 μL of reaction working solution (mix well 120 μL of coenzyme working solution and 40 μL of substrate working solution). The

reaction working solution should be prepared on spot, and use up within 1 h.

⑥ The preparation of 500 μmol/L standard solution：

Dissolve a vial of standard with 1.6 mL double distilled water. Store at 2-8 ℃ for 3 days protected from light.

⑦ The preparation of standard curve：

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

Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 150, 200, 300, 400, 450, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**150**	**200**	**300**	**400**	**450**	**500**
500 μmol/L Standard solution (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.

Tissue sample:

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

② Wash tissue in cold normal saline (0.9% NaCl).

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

④ Centrifuge at 10000×g for 15 minutes 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 kidney tissue homogenate	4-8
10% Mouse kidney tissue homogenate	4-8
10% Mouse liver tissue homogenate	4-8
10% Mouse lung tissue homogenate	4-8
Mouse serum	4-8
Rat serum	4-8
Human serum	4-8
Rat plasma	4-8

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Avoid bubbles when adding reaction working solution.

② The reaction process should be with shading light.

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.

② Add 160 μL of reaction working solution, 20 μL of chromogenic agent to each well respectively.

③ Mix fully with microplate reader for 3 s and stand at room temperature with shading light for 5 min. Measure the OD value of sample well at 450 nm with microplate reader, recorded as A_1.

④ Incubate at 37°C for 45 min with shading light. Measure the OD value of sample well and standard well at 450 nm with microplate reader, recorded as A_2, $\triangle A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. 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.

$$\underset{\text{(U/L)}}{\text{ALDH activity}} = (\Delta A_{450} - b) \div a \div T \times f$$

2. Tissue sample:

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

$$\underset{\text{(U/gprot)}}{\text{ALDH activity}} = (\Delta A_{450} - b) \div a \div T \div C_{pr} \times f$$

[Note]

ΔA_{450}: The change OD values of sample well (A_2-A_1).

T: The time of incubation reaction, 45 min.

C_{pr}: The concentration of protein in tissue, 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 (U/L)	0.56	2.7	8.50
%CV	3.5	3.0	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.56	2.7	8.50
%CV	6.8	7.5	7.6

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc.（μmol/L）	135	264	425
Observed Conc.（μmol/L）	136.4	253.4	425.0
Recovery rate (%)	101	96	100

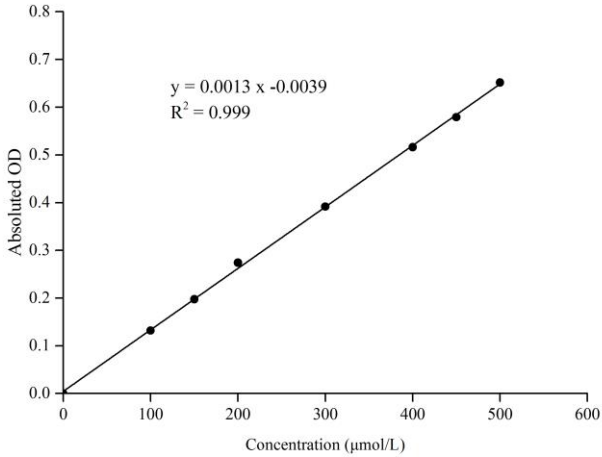
Sensitivity

The analytical sensitivity of the assay is 0.01 U/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 OD 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**	**100**	**150**	**200**	**300**	**400**	**450**	**500**
OD value	0.056	0.184	0.253	0.333	0.459	0.576	0.633	0.708
	0.052	0.188	0.25	0.323	0.432	0.564	0.633	0.703
Average OD	0.054	0.186	0.252	0.328	0.446	0.570	0.633	0.706
Absoluted OD	0.000	0.132	0.198	0.274	0.392	0.516	0.579	0.652



Appendix II Example Analysis

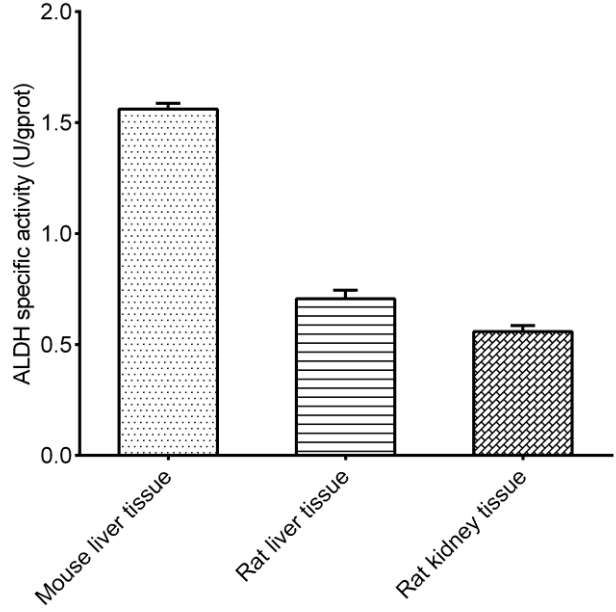
Example analysis:

For mouse liver tissue, take 20 μL of 10% mouse liver tissue homogenate, dilute for 4 times, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0013 x - 0.0039, the OD value of the sample A_1 is 0.131, the OD value of the sample A_2 is 0.285, the concentration of protein in sample is 7.03 gprot/L, and the calculation result is:

ALDH activity (U/gprot) = (0.285 – 0.131 + 0.0039) ÷ 0.0013 ÷ 45 ÷ 7.03 × 4 = 1.53 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 7.03 gprot/L, dilute for 4 times), 10% rat liver tissue homogenate (the concentration of protein is 5.65 gprot/L, dilute for 4 times) and 10% rat kidney tissue homogenate (the concentration of protein is 7.35 gprot/L, dilute for 4 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.