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

Catalog No：E-BC-K763-M

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

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.29-248 U/L

Elabscience®Alcohol Dehydrogenase (ADH) 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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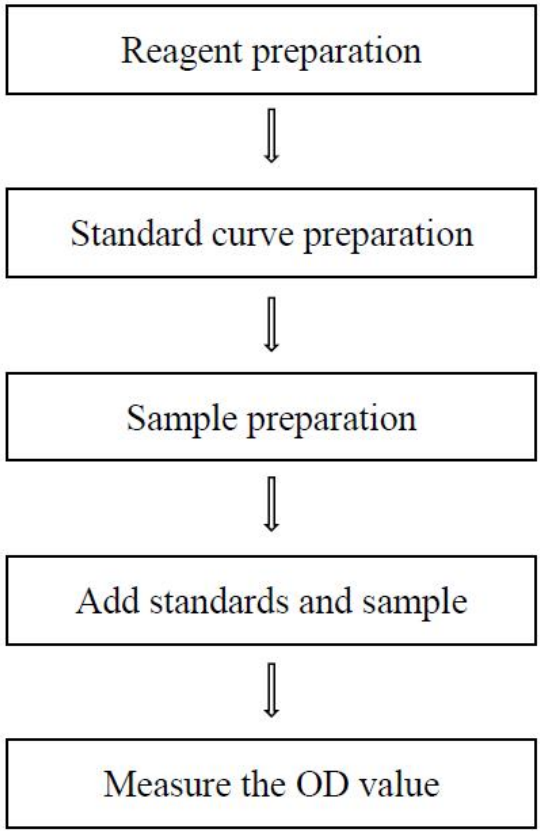
Assay summary



Intended use

This kit can measure alcohol dehydrogenase (ADH) activity in serum (plasma) and animal tissue samples.

Detection principle

Alcohol dehydrogenase (ADH), an ehanol oxidoreductase, is the key enzyme of short chain alcohol metabolism in organisms. It catalyzes the reversible conversion between ethanol and acetaldehyde and plays an important role in many physiological processes. In mammals, ADH is mainly produced in the liver, and liver damage leads to the release of ADH into serum. The changes of serum ADH activity are closely related to such phenomena as alcoholic liver cell injury, hepatitis, and liver cirrhosis. ADH catalyzes the oxidative dehydrogenation of ethanol. 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 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	Substrate	1.5 mL × 1 vial	1.5 mL × 2 vials	-20℃, 12 months
Reagent 2	Coenzyme	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months
Reagent 3	Coenzyme Diluent	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months
Reagent 4	Buffer Solution	15 mL×1 vial	30 mL×1 vial	-20℃, 12 months
Reagent 5	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months
	Microplate	48 wells	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)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of coenzyme working solution 1：

Dissolve one vial of coenzyme with 400 μL of coenzyme diluent, mix well to dissolve. Aliquoted storage at -20°C for 7 days.

③ Preparation of coenzyme working solution 2：

Before testing, please prepare sufficient coenzyme working solution 2 according to the test wells. For example, prepare 200 μL of coenzyme working solution 2 (mix well 2 μL of coenzyme working solution 1 and 198 μL of buffer solution, mix well. Keep coenzyme working solution 2 on ice during use. The working solution should be prepared on spot and used up within 0.5 h.

④ Preparation of reaction working solution：

For each well, prepare 160 μL of reaction working solution (mix well 140 μL of coenzyme working solution 2 and 20 μL of substrate). The working solution

should be prepared on spot and used up within 0.5 h.

⑤ Preparation of 5 mmol/L standard stock solution：

Dissolve one vial of standard with 1 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 5 days.

⑥ Preparation of 250 μmol/L standard solution：

Before testing, please prepare sufficient 250 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 250 μmol/L standard solution (mix well 50 μL of 5 mmol/L standard stock solution and 950 μL of buffer solution). The standard solution should be prepared on spot and used up within 6 h.

⑦ The preparation of standard curve：

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

Dilute 250 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 75, 100, 150, 175, 200, 250 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**50**	**75**	**100**	**150**	**175**	**200**	**250**
250 μmol/L standard (μL)	0	40	60	80	120	140	160	200
Buffer solution (μL)	200	160	140	120	80	60	40	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℃ 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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 lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat liver tissue homogenate	2-4
10% Rat heart tissue homogenate	1-3
10% Mouse liver tissue homogenate	2-4
10% Rat brain tissue homogenate	1-3
Mouse serum	1
Porcine serum	1
Human serum	1
Dog serum	1

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.
③ The prepared coenzyme working solution should be used within 0.5 h.

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, and 40 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 2 min. Measure the OD value of sample well at 450 nm with microplate reader, recorded as A_1.
④ Incubate at 37℃ for 15 min with shading light.
⑤ 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 ADH in 1 L liquid sample per 1 minute that hydrolyze the ethanol to produce 1 μmol NADH at 37°C is defined as 1 unit.

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

2. Tissue sample:

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

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

[Note]

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

T: The time of incubation reaction, 15 min.

Cpr: The concentration of protein in sample, 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)	8.40	56.50	138.00
%CV	4.5	4.1	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 (U/L)	8.40	56.50	138.00
%CV	4.6	4.0	4.9

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （μmol/L）	62	135	189
Observed Conc. （μmol/L）	63.9	133.7	196.6
recovery rate(%)	103	99	104

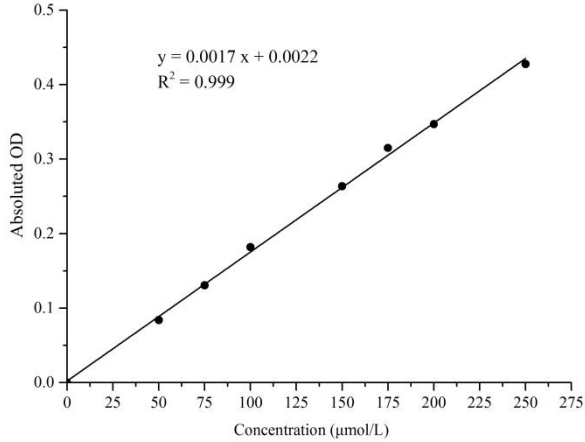
Sensitivity

The analytical sensitivity of the assay is 0.29 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	50	75	100	150	175	200	250
Average OD	0.063	0.147	0.194	0.245	0.327	0.378	0.410	0.491
Absoluted OD	0.000	0.084	0.131	0.182	0.264	0.315	0.347	0.428



Appendix II Example Analysis

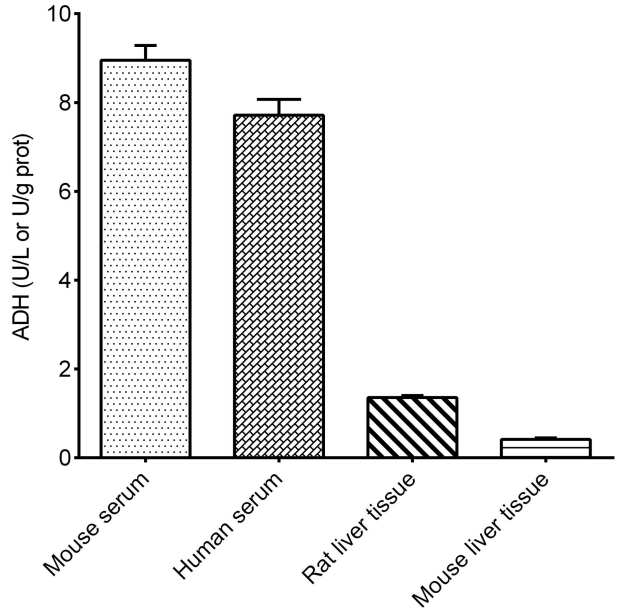
Example analysis:

For 10% rat liver tissue homogenate, dilute for 2 times, and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0017 x + 0.0022, the average OD value of the sample (A_1) is 0.193, the average OD value of the sample (A_2) is 0.478, the concentration of protein in sample is 8.14 gprot/L, and the calculation result is:

ADH activity (U/gprot) = (0.478 – 0.193 – 0.0022) ÷ 0.0017 ÷ 15 ÷ 8.14 × 2 = 2.72 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 6.58 gprot/L, dilute for 2 times), 10% mouse liver tissue homogenate (the concentration of protein is 7.08 gprot/L, dilute for 2 times), mouse serum and human serum 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.