PRODUCT INFORMATION & MANUAL

alcohol dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24463

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

alcohol dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24463

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.29 U/L

Detection range: 0.29-248 U/L

Average intra-assay CV (%): 4.2

Average inter-assay CV (%): 4.5

Average recovery rate (%): 102

▲ 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 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	Specification	Storage
Reagent 1	Substrate	1.5 mL × 2 vials	-20℃ , 12 months
Reagent 2	Coenzyme	Powder × 2 vials	-20℃ , 12 months
Reagent 3	Coenzyme Diluent	1 mL × 1 vial	-20℃ , 12 months
Reagent 4	Buffer Solution	30 mL×1 vial	-20℃ , 12 months
Reagent 5	Chromogenic Agent	6 mL × 1 vial	-20℃ , 12 months shading light
Reagent 6	Standard	Powder × 2 vials	-20℃ , 12 months
	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

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

Reagents:

Normal saline (0.9% NaCl)

⚠ 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

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.

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 400 μL reagent 3. The prepared solution can be stored at -20°C for 7 days.
3. Preparation of coenzyme working solution:

   Mix the reagent 2 working solution and reagent 4 at the ratio of 1:99 fully and preserve it on ice for detection. Prepare the fresh needed amount before use and the prepared solution should be used within 0.5 h.
4. Preparation of reaction working solution:

   Mix the coenzyme working solution and reagent 1 at the ratio of 7:1 fully and preserve it on ice for detection. Prepare the fresh needed amount before use and the prepared solution should be used within 0.5 h.
5. Preparation of 5 mmol/L standard stock solution:

   Dissolve a vial of reagent 6 powder with 1 mL reagent 4. The prepared solution can be stored at -20°C for 5 days.
6. Preparation of 250 μmol/L standard solution:

   Mix the 5 mmol/L standard stock solution and reagent 4 at the ratio of 1:19 fully. Prepare the fresh needed amount before use and the prepared solution should be used within 6 h.

▲ Sample preparation

1. Serum and plasma samples:

   Detect the sample directly.

2. Tissue sample:

   Accurately weigh the tissue, add normal saline 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.

▲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 (0.29-248 U/L).

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).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

1. The preparation of standard curve

Dilute 250 μmol/L standard solution with reagent 4 to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 75, 100, 150, 175, 200, 250 μmol/L.

Number	Standard concentrations (μmol/L)	250 μmol/L Standard (μL)	Reagent 4 (μL)
A	0	0	200
B	50	40	160
C	75	60	140
D	100	80	120
E	150	120	80
F	175	140	60
G	200	160	40
H	250	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.

(2) Add 160 μL of reaction working solution, and 40 μL of regent 5 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, $\Delta A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(Standard)}$).

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	20	
Sample (μL)		20
Reaction working solution (μL)	160	160
Regent 5 (μL)	40	40
Mix fully and stand at room temperature with shading light for 2 min. Measure the OD value of sample well at 450 nm, recorded as A_1.		
Incubate at 37℃ for 15 min with shading light. Measure the OD value of sample well and standard well at 450 nm, recorded as A_2, $\Delta A = A_2 - A_1$.		

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 :

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.

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

2. 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.

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

Note:

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

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

T: The time of incubation reaction, 15 min.

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

f: Dilution factor of sample before tested.

Appendix I Data

▲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