TECHNICAL MANUAL

Alcohol Dehydrogenase (ADH) Activity Assay Kit

- **SKU CODE:** MAES0216
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure absorbance at 450 nm
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

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

3. Detection principle

Alcohol dehydrogenase (ADH), an ethanol 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. Changes in serum ADH activity are closely related to 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, transfers electrons to WST-8 to produce a yellow product. The activity of ADH can be calculated by measuring the change in absorbance value at 450 nm.

4. 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°C, 12 months
Reagent 2	Coenzyme	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months
Reagent 3	Coenzyme Diluent	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months
Reagent 4	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 5	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

6. 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. Aliquot and store at -20°C for 7 days.
3. **Preparation of coenzyme working solution 2::** Before testing, 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 fresh 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 fresh 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. Aliquot and store at -20°C for 5 days.

6. **Preparation of 250 μmol/L standard solution::** Before testing, 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 fresh 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.

7. Sample preparation

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 one 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 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of sample:

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

- 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Avoid bubbles when adding reaction working solution.

2. The reaction process should be protected from light.

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

9. 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 protected from light for 2 min. Measure the OD value of sample well at 450 nm with microplate reader, recorded as A1.

4. Incubate at 37°C for 15 min protected from light.

5. Measure the OD value of sample well and standard well at 450 nm with microplate reader, recorded as A2, ΔA = A2 - A1. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of A2(standard).

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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 hydrolyzes ethanol to produce 1 μmol NADH at 37°C is defined as 1 unit.

ADH activity (U/L) = (ΔA_450 - b) ÷ a ÷ T × f

2. Tissue sample:

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

ADH activity (U/gprot) = (ΔA_450 - b) ÷ a ÷ T ÷ C_{pr} × f

[Note]

ΔA_450: The change in OD values of sample well (A_2-A_1).

T: The time of incubation reaction, 15 min.

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

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	8.40	56.50	138.00
%CV	4.6	4.0	4.9

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 102%.

Recovery

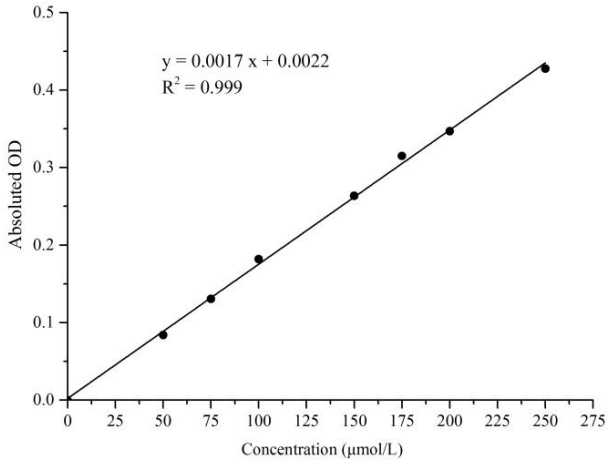
Standard	Expected Conc. (µmol/L)	Observed Conc. (µmol/L)	Recovery rate(%)
Standard 1	62	63.9	103
Standard 2	135	133.7	99
Standard 3	189	196.6	104

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.063	0.000
50	0.147	0.084
75	0.194	0.131
100	0.245	0.182
150	0.327	0.264
175	0.378	0.315
200	0.410	0.347
250	0.491	0.428

12. Appendix II Example Analysis

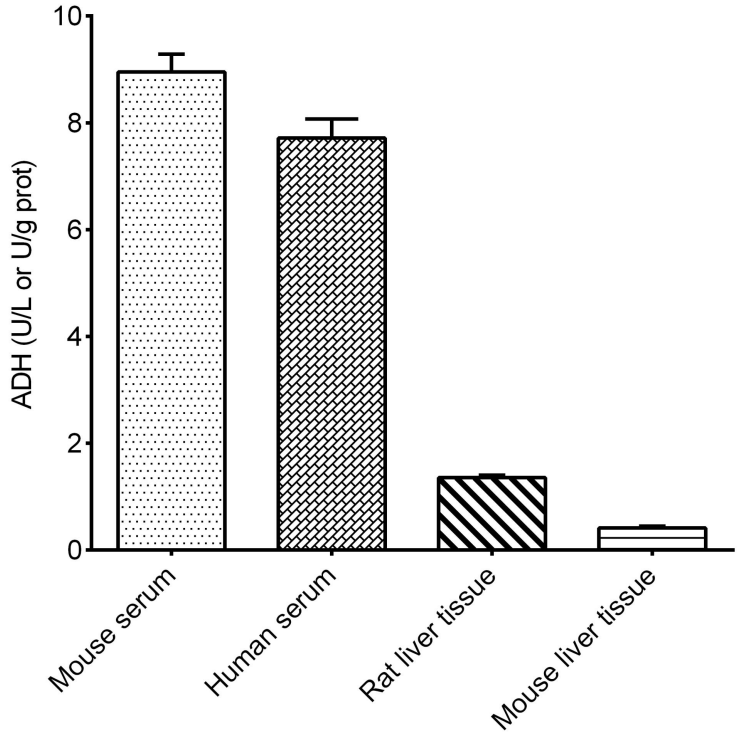
Example analysis:

For 10% rat liver tissue homogenate, dilute 2-fold, and perform the assay according to the operation protocol. The results are as follows:

Standard curve: y = 0.0017x + 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 2-fold), 10% mouse liver tissue homogenate (the concentration of protein is 7.08 gprot/L, dilute 2-fold), mouse serum and human serum according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.