AssayGenie

TECHNICAL MANUAL

Aldehyde Dehydrogenase (ALDH) Fluorometric Activity Assay Kit

- **SKU CODE:** MAES0221
- **SIZE:** 96 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
- Add reaction working solution
- Add chromogenic working solution
- Measure fluorescence intensity

2. Intended use

This kit can measure aldehyde dehydrogenase (ALDH) activity in serum (plasma), animal tissue, and cell samples.

3. Detection principle

The main pathway of alcohol metabolism is oxidation by alcohol dehydrogenase (ADH) to acetaldehyde, followed by NADH-dependent acetaldehyde dehydrogenase (ALDH) oxidation to acetic acid.

The detection principle of this kit is based on the substrate being transformed by aldehyde dehydrogenase to convert NAD+ into NADH. NADH reacts with the fluorescent probe under enzymatic action to form a fluorescent substance. ALDH activity can be calculated by measuring fluorescence intensity at an excitation wavelength of 535 nm and emission wavelength of 587 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL x 1 vial	-20°C, 12 months
Reagent 2	Coenzyme	Powder x 2 vials	-20°C, 12 months, protected from light
Reagent 3	Substrate	0.3 mL x 1 vial	-20°C, 12 months, protected from light

Item	Component	Size (96 T)	Storage
Reagent 4	Chromogenic Agent	0.2 mL x 1 vial	-20°C, 12 months, protected from light
Reagent 5	Enzyme Reagent	Powder x 2 vials	-20°C, 12 months, protected from light
Reagent 6	Standard	Powder x 2 vials	-20°C, 12 months, protected from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments: Micropipette, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of coenzyme working solution:** Dissolve one vial of coenzyme with 1 mL of double distilled water and mix well. Store at 2-8°C for 2 days protected from light.
3. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the number of test wells. For example, to prepare 1928 µL of reaction working solution, thoroughly mix 1770 µL of buffer solution, 150 µL of coenzyme working solution, and 8 µL of substrate. The reaction working solution should be prepared fresh and used within 6 hours.
4. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 100 µL of double distilled water, mix well, and keep on ice for use. Store at 2-8°C for 1 day protected from light.
5. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the number of test wells. For example, to prepare 301 µL of chromogenic working solution, thoroughly mix 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 fresh and used within 6 hours.

6. **Preparation of 300 µmol/L standard:** Dissolve one vial of standard with 1.6 mL of double distilled water and mix well to dissolve completely. Store at 4°C for 2 days protected from light.
7. **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 fresh.
8. **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 serial concentrations. The recommended dilution gradient is: 0, 0.6, 0.9, 1.2, 1.8, 2.4, 2.7, 3 µmol/L.

7. Sample preparation

Serum and plasma: Detect directly. If not analyzed on the same day, 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 to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

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 an ultrasonic cell disruptor at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of samples:

The recommended dilution factors for different samples are as follows (for reference only):

- 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 other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

Avoid bubbles when adding reaction working solution.

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. Control well: Add 20 µL of sample to the corresponding wells.
2. Add 140 µL of reaction working solution to the standard and sample wells. Add 140 µL of buffer solution to the control wells.
3. Add 20 µL of chromogenic working solution to each well.
4. Mix thoroughly with microplate reader for 3 seconds and incubate at room temperature protected from light for 5 minutes. Measure the fluorescence intensity of each well at excitation wavelength 535 nm and emission wavelength 587 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute fluorescence value of standards as the y-axis and corresponding concentration as the x-axis. 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 hydrolyzes acetaldehyde to produce 1 μmol NADH at 37°C is defined as 1 unit.

ALDH activity (U/g_{prot}) = (F_{sample} - $F_{control}$ - b) ÷ a ÷ T ÷ C_{pr} × f

2. Serum (plasma) sample:

Definition: The amount of ALDH in 1 L liquid sample per 1 minute that hydrolyzes acetaldehyde to produce 1 μmol NADH at 37°C is defined as 1 unit.

ALDH activity (U/L) = (F_{sample} - $F_{control}$) ÷ a ÷ T × f

[Note]

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

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

T: The incubation reaction time, 5 min.

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

f: Dilution factor of sample before test.

Intra-assay Precision

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

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

Recovery

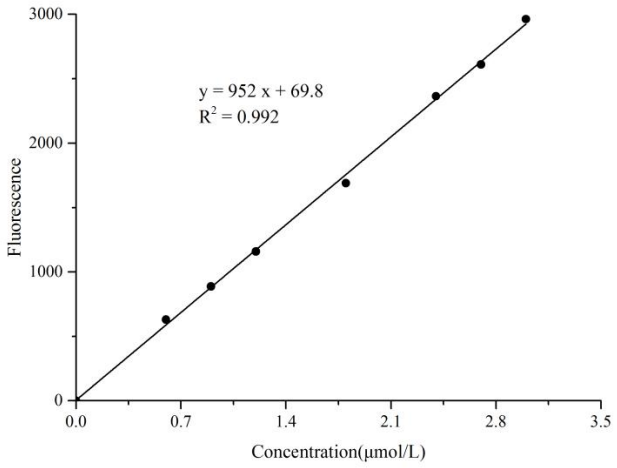
Standard 1	Standard 2	Standard 3
0.8	1.5	2.5
0.8	1.5	2.5
101	99	100

Sensitivity

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

Standard curve data

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



11. Example Analysis

Example analysis:

For mouse liver tissue, take 40 µL of 10% mouse liver tissue homogenate, diluted 180 times, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 952x + 69.8

Average fluorescence value of control: 413

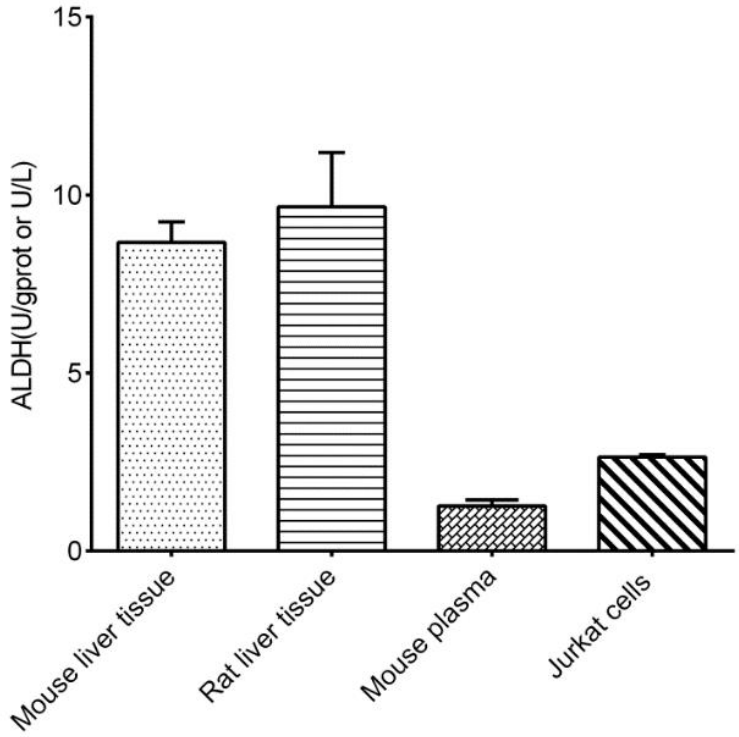
Average fluorescence value of sample: 1537

Protein concentration in sample: 3.51 g_{prot}/L

Calculation result:

ALDH activity (U/g_{prot}) = (1537 - 413 - 69.8) ÷ 952 ÷ 5 ÷ 3.51 × 180 = 11.36 U/g_{prot}

Detect 10% mouse liver tissue homogenate (protein concentration: 3.51 g_{prot}/L, diluted 180 times), 10% rat liver tissue homogenate (protein concentration: 11.95 g_{prot}/L, diluted 180 times), mouse plasma (diluted 3 times), and Jurkat cells (protein concentration: 0.3 g_{prot}/L, diluted 10 times) according to the protocol. The results are as follows:



12. 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 pretest 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.