



Ver.260401

Aldehyde Dehydrogenase (ALDH) Activity Assay Kit

BC6612-01 (50T/48S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Acetaldehyde dehydrogenase (ALDH) is a type of aldehyde dehydrogenase that can catalyze the rapid dehydrogenation of toxic aldehydes such as acetaldehyde, n-butyraldehyde, cinnamaldehyde, and benzaldehyde, oxidizing these aldehydes into carboxylic acids. It eliminates harmful aldehydes and reduces lipid peroxidation reactions, being considered as a detoxifying agent for reactive oxygen species in biological organisms. Acetaldehyde dehydrogenase not only converts and metabolizes aldehydes harmful to organisms but also has extensive research applications in molecular biology and the detection of related diseases.

Acetaldehyde dehydrogenase catalyzes the conversion of acetaldehyde and NAD^+ into acetic acid and NADH. By measuring the change in absorbance of NADH at 340nm, the activity of acetaldehyde dehydrogenase can be calculated.

Kit components

Reagent	Volume	Storage
Extraction Solution	60 mL	2-8°C
Powder I	20 mL	2-8°C
Reagent II	Powder×2	-20°C
Reagent III	1.2 mL	2-8°C
Reagent IV	2 mL	2-8°C
Reagent V	20 mL	2-8°C

Note:

Reagent II: Dissolve with 3 mL of distilled water one of the bottle before using, and unused liquid can be stored at -20°C for 4 weeks after subassembly to avoid repeated freezing and thawing.

Reagent IV: Reagent IV is toxic, pay attention to protection during the experiment;

Preparation of working solution: Before use, the working liquid was prepared according to the sample size in the ratio of Reagent I: Reagent II: Reagent III: Reagent IV =300 μL : 100 μL : 20 μL : 30 μL (about 1T) mixed for standby, ready for use.

Reagents and Equipment Required but Not Provided

Ultraviolet spectrophotometer, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, 1mL quartz cuvette, mortar /homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol

I. Sample Preparation.

1. Bacteria/Cells

According to the number of bacteria or cells (10^4): the volume of Extraction Solution (mL) is 500-1000:1 to prepare (it is recommended that add 1 mL of extraction solution to 500 million of cells). Bacteria/cells is split by ultrasonication (power 300W, ultrasonic 3 seconds, interval 7 seconds, total time 3 min). Centrifuge at 10000 g and 4°C for 20 minutes. Take the supernatant on ice for test.

2. Tissue:

According to the tissue mass (g): the volume of the Extract solution (mL) is 1:5~10 to prepare (it is recommended that add 1 mL of Extract solution to 0.1 g of tissue). Homogenate on ice. Centrifuge at 10000 g and 4°C for 20 minutes. Take the supernatant on ice for test.

3. Serum (plasma) sample:

Detect sample directly. If the liquid is turbid, it needs to be measured after centrifugation).

II. Determination Procedures

- Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.
- Preheat working solution at 37°C for 10 minutes.
- Operation table:

Reagent(μL)	Blank tube (A _B)	Test tube (A _T)
Sample	-	200
Distilled water	200	-
Working Solution	450	450
Reagent V	350	350

The above reagents are added into the 1 mL quartz cuvette in sequence. Mix thoroughly. Measure the absorbance A₁ at 340 nm for 1 minutes. Put it in a water bath or incubator at 37°C for 30 minutes. Take it out and dry it quickly, and then measure the absorption value A₂ at 31 minutes. $\Delta A_T = A_{2T} - A_{1T}$. $\Delta A_B = A_{2B} - A_{1B}$. $\Delta A = \Delta A_T - \Delta A_B$. Blank tube just need to test once or twice.

Note

Every Test tube need set a contrast tube. Different samples of crude enzyme solution can be added to different contrast tubes and then heat in boiled water for 5 minutes.

III. ALDH Calculations

1. Calculate by sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per minute in the reaction system every milligram tissue protein.

$$\text{ALDH activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div (C_{pr} \times V_{SA}) \div T$$

$$= 26.795 \times \Delta A \div C_{pr}$$

2. Calculate by sample mass::

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per minute in the reaction system every gram tissue mass.

$$\text{ALDH activity (U/g mass)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div (V_{SA} \times W \div V_E) \div T$$

$$= 26.795 \times \Delta A \div W$$

3. Calculate by the number of bacteria or cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per minute in the reaction system every 10⁶ cells or germ.

$$\text{ALDH activity (U/10}^6\text{cell)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div (V_{SA} \times V_E \div N) \div T$$

$$= 26.795 \times \Delta A \div N$$

4. Calculate by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per minute in the reaction system every milliliter liquid.

$$\text{ALDH activity (U/mL)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div V_{SA} \div T = 26.795 \times \Delta A$$

ϵ : NADH molar extinction coefficient, 6.22×10³ L/mol/cm;

d : Light path of cuvette, 1 cm;

V_{RT} : Total reaction volume, 0.001 L;

V_{SA} : Sample volume, 0.2 mL;

V_E : Extract solution volume, 1 mL;

T : Reaction time, 30 min;

C_{pr} : Protein concentration, mg/mL;

N : Number of cells, count by 10⁶;

W : Sample mass, g;

10⁹: unit conversion factor, 1 mol=10⁹ nmol.

Note:

1. The blank tube is the test hole for testing the quality of each reagent component. Under normal circumstances, the OD value should not exceed 0.3, and the change should not exceed 0.01.
2. When the ΔA is greater than 1.0, it is recommended to measure after dilution. When ΔA is less than 0.01, the reaction time can be prolonged to 60 minutes or longer for determination.

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