CheKine™ Micro Acetaldehyde Dehydrogenase (ALDH) Activity Assay Kit

Cat #: KTB3032 Size: 48 T/96 T

	Micro Acetaldehyde Dehydrogenase (ALDH) Activity Assay Kit		
REF	Cat #: KTB3032	LOT	**Lot #: Refer to product label**
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Acetaldehyde dehydrogenase (EC 1.2.1.10, ALDH) is a kind of aldehyde dehydrogenase, which is widely present in various animals, plants and microorganisms. The main effect is the oxidation of acetaldehyde to acetic acid, which plays a major role in alcohol metabolism. In humans and many animals, mitochondrial aldehyde dehydrogenase (MDA) can convert alcohols that are harmful to organisms, so it has attracted great attention in the research of cellular detoxification. At the same time, aldehyde dehydrogenase has been widely used in molecular biology and the detection of related diseases. In the presence of coenzyme Ⅰ, acetaldehyde dehydrogenase catalyzes the conversion of acetaldehyde and NAD^+ to acetate and NADH, and the absorbance value at 340 nm increases. The activity of acetaldehyde dehydrogenase can be calculated by measuring the absorbance value at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	12.5 mL	25 mL	4℃
Reagent Ⅱ	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
·Water bath, Ice machine, Centrifuge
·Deionized water
·Homogenizer (for tissue samples)

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Reagent Preparation

Extraction Buffer： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅱ： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃,protected from light

Working Reagent： Prepared before use; according to the number of samples, the samples were mixed in a ratio of Reagent I: Reagent II=200 μL:10 μL, mixed thoroughly, and used as prepared.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1.Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 20 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2.Cells: Collect 5×10^6 cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 3 min (power 300W, ultrasonic 3 s, interval 7 s,the total time was 3 min). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3.Plasma, Serum or other Liquid samples: Direct detection.If the solution was cloudy, the supernatant was taken after centrifugation for determination.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1.Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet spectrophotometer was returned to zero with deionized water.

2.The Working Reagent was preheated at 37°C for more than 10 min.

3.Sample measurement.(The following operations are operated in the 96-well UV plate or microquartz cuvette):

Reagent	Test Well（μL）
Sample supernatant	20
Working Reagent	180

The mixture was mixed thoroughly and the initial absorbance value A_1 was measured at 340 nm. After incubation at 37℃ for 30 min, the absorbance value A_2 was measured at 340 nm, and $\Delta A=A_2-A_1$ was calculated.

Note: Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre-experiment. If ΔA is less than 0.01, the sample size can be appropriately increased or the reaction time can be appropriately prolonged (for example, 60 min). If ΔA is greater than 1.0, the sample can be further diluted with Extraction Buffer, the calculation result is multiplied by the dilution, or the sample size for extraction is reduced.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula as below

（1）Calculated by sample protein concentration:

Unit definition: the amount of enzyme catalyzing the reduction of 1 nmol NAD^+ per min per mg of protein is one unit of enzyme

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activity.

ALDH (U/mg prot)=$\Delta A \div (\varepsilon \times d) \times V_{Reaction\ volume} \div (V_{sample} \times Cpr) \div T$=**$107.17 \times \Delta A \div Cpr$**

(2) Calculated by fresh weight of samples:

Unit definition: the amount of enzyme catalyzing the reduction of 1 nmol NAD^+ per min per mg of protein is one unit of enzyme activity.

ALDH (U/g fresh weight)=$\Delta A \div (\varepsilon \times d) \times V_{Reaction\ volume} \div (V_{sample} \times W \div V_{Total\ sample}) \div T$=**$107.17 \times \Delta A \div W$**

(3) Calculated by cells:

Unit definition: the amount of enzyme catalyzing the reduction of 1 nmol NAD^+ per min per mg of protein is one unit of enzyme activity.

ALDH (U/10^4 cell)=$\Delta A \div (\varepsilon \times d) \times V_{Reaction\ volume} \div (V_{sample} \times 500 \div V_{Total\ sample}) \div T$=**$107.17 \times \Delta A \div 500$**

(4) Calculated by volume of liquid samples:

Unit definition: the amount of enzyme catalyzing the reduction of 1 nmol NAD^+ per min per mg of protein is one unit of enzyme activity.

ALDH (U/mL)= $\Delta A \div (\varepsilon \times d) \times V_{Reaction\ volume} \div V_{sample} \div T$=**$107.17 \times \Delta A$**

ε: NADH molar extinction coefficient, 6.22×10^{-3} L/μmol/cm; d: optical diameter of 96-well plate, 0.5 cm; $V_{Reaction\ volume}$: Total volume of the reaction system, 0.2 mL; V_{sample}: Added sample volume to the reaction system, 0.02 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL; Cpr: Sample protein concentration, mg/mL; W; Sample weight, g; T: Reaction time , 30 min.

Precautions

1. Reagent II is toxic; during experimentation, please wear protective measures such as masks and gloves.

Typical Data

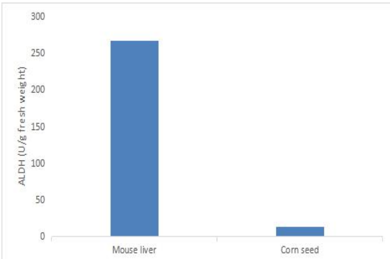

Figure 1. Determination of acetaldehyde dehydrogenase activity in Mouse liver and Corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase (α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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