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Aldehyde Dehydrogenase(ALDH) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: AK0268

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃;

Reagent I: Liquid 10 mL×1, store at 4℃;

Reagent II: Powder×1, store at -20℃ and protect from light. Add 3 mL of distilled water when the solution will be used. The rest of reagent store at -20℃; It can also be prepared in proportion when the solution will be used;

Reagent III: Liquid 0.5 mL×1, store at 4℃ and protect from light;

Reagent IV: Liquid 1 mL×1, store at 4℃;

Reagent V: Liquid 2 mL×1, store at 4℃ .

Product Description:

Aldehyde dehydrogenase (EC 1.2. 1. 10) is a kind of aldehyde dehydrogenase. It widely exists in various animals, plants and microorganisms. In the presence of coenzyme I, it can catalyze the dehydrogenation of some primary or secondary alcohols including ethanol, aldehydes or ketones. In humans and many animals, mitochondrial acetaldehyde dehydrogenase can transform harmful alcohols. So in the study of cell detoxification, glyoxal dehydrogenase is highly concerned; Aldehyde dehydrogenase is widely used in molecular biology and detection of related diseases.

Acetaldehyde dehydrogenase catalyzes the conversion of acetaldehyde and NAD^+ to acetic acid and NADH. The activity of aldehyde dehydrogenase can be calculated by the change of absorbance value of NADH at 340 nm.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath, adjustable pipette, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ice and distilled water.

Protocol

I. Preparation:

1. Tissue:

According to the tissue weight (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℃ for 20 minutes. Take the supernatant on ice for test.

2. Cells or bacterial

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

3. Liquid: detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 min, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Preheat Reagent I in 37℃ (mammal) or 25℃ (other species) for 15 min.

3. Operation table:

Reagent Name (μL)	Blank tube (A_B)	Test tube (A_T)
Sample		40
Distilled water	100	60
Reagent I	60	60
Reagent II	20	20
Reagent III	4	4
Reagent IV	6	6
Reagent V	10	10
The above reagents are added into the micro quartz cuvette/96 well flat-bottom UV plate in sequence. Mix thoroughly. Measure the absorbance A1 at 340 nm for 30s. Put it in a water bath or incubator at 37℃(mammal) or 25℃ (other species) for 1 min (if the microplate reader has the function of temperature control, adjust the temperature to 37℃ or 25℃). Take it out and dry it quickly, and then measure the absorption value A2 at 90s. $\Delta A_T=A2_T-A1_T$. $\Delta A_B=A2_B-A1_B$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube just need to test once or twice.		

III. ALDH Calculation:

a. Micro quartz cuvette

1) 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.

$ALDH(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{SA})\div T\times 10^9= 804\times\Delta A\div Cpr$

2) Sample weight:

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 weight.

$ALDH(U/g\ weight)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T\times 10^9=804\times\Delta A\div W$

3) Cells or germ

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 10^4 cells or germ.

$ALDH(U/10^4\ cell)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(cells\ (million)\times V_{SA}\div V_E)\div T\times 10^9=804\times\Delta A\div cells\ (million)$

4) 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.

$ALDH(U/mL) = \Delta A \div (\varepsilon \times d) \times V_{RT} \div V_{SA} \div T \times 10^9 = 804 \times \Delta A$

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
V_{RT}: Total reaction volume, 0.0002 L;
V_{SA}: Sample volume, 0.04 mL;
V_E: Extract solution volume, 1 mL;
T: Reaction time, 1 min;
Cpr: Protein concentration, mg/mL;
W: Sample weight, g.

b. 96 well flat-bottom plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well flat- bottom UV plate.

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 5 min or 10 min for determine.

Experimental example:

1. Take 0. 1g of mouse kidney and add 1 mL of Extract solution to grind it in homogenate, clean it up, and then follow the measurement procedure. Calculate the enzyme activity according to the sample quality ALDH enzyme activity (U/g mass) =804 × ΔA ÷ W= 804 × 0.6864 ÷ 0. 1=5518.656 U/g mass.

Recent Product Citations：

[1] Tongmeng Jiang,Jinmin Zhao,Shan Yu,et al. Untangling the response of bone tumor cells and bone forming cells to matrix stiffness and adhesion ligand density by means of hydrogels. Biomaterials. January 2019;188:130- 143.(IF5.452)

[2] Chong Li, Shi Gao, Xiaotong Li,et al. Efficient metabolic evolution of engineered Yarrowia lipolytica for succinic acid production using a glucose-based medium in an in situ fibrous bioreactor under low-pH condition. Biotechnology for Biofuels. August 2018;(IF5.452)

[3] Yufei He,Xiaoyan Ci,Ying Xi,et al. Untangling the response of bone tumor cells and bone forming cells to matrix stiffness and adhesion ligand density by means of hydrogels. Biomaterials. September 2018;(2019)188:130- 143.(IF8.806)

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AK0262/AK0261	Alcohol Dehydrogenase(ADH) Activity Assay Kit