Aldehyde Dehydrogenase(ALDH) Activity Assay Kit

Note: The reagents of this product have changed, please operate in strict accordance with the instructions.

Operation Equipment: Spectrophotometer

Cat No: BC0750

Size: 50T/48S

Components:

Extract solution: Liquid 60 mL×1, Storage at 2-8℃;

Reagent I: Liquid 20 mL×1, Storage at 2-8℃;

Reagent II: Powder×2, Storage at -20℃ and protect from light. Before use, take a bottle of Reagent II and add 3 mL of distilled water to dissolve it. Unused reagents can be stored in aliquots at -20℃ for 4 weeks, avoiding repeated freezing and thawing;

Reagent III: Liquid 1.2 mL×1, Storage at 2-8℃;

Reagent IV: Liquid 2 mL×1, Storage at 2-8℃. Reagent IV is toxic, pay attention to protection during the experiment;

Reagent V: Liquid 20 mL×1, Storage at 2-8℃.

Working Solution: According to the amount of Reagent I: Reagent II: Reagent III: Reagent IV =300μL: 100μL: 20μL: 30μL (about 1T) mixed for standby, ready for use.

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, aldehydes or ketones, including ethanol. 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, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, 1 mL quartz cuvette, mortar /homogenizer/ sonicator, 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 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 3s, interval 7s, total time 3 min). Centrifuge at 10000 g and 4℃ for 20 minutes. Take the supernatant on ice for test.
3. Serum or other liquids: detect directly. (If the liquid is turbid, it needs to be measured after centrifugation)

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat Working Solution in 37℃ (mammal) or 25℃ (other species) for 10 minutes.
3. Operation table:

Reagent Name (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℃(mammal) or 25℃ (other species) for 30 minutes. Take it out and dry it quickly, and then measure the absorption value A2 at 31minutes. $\Delta A_T=A2_T-A1_T$. $\Delta A_B=A2_B-A1_B$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube just need to test once or twice.		

III. ALDH Calculation:

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) $=\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(Cpr\times V_{SA})\div T=26.795\times\Delta A\div Cpr$
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) $=\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(V_{SA}\times W\div V_E)\div T=26.795\times\Delta A\div W$
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 106 cells or germ.
   ALDH (U/10^6 cell) $=\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(V_{SA}\div V_E\times N)\div T=26.795\times\Delta A\div N$
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) =ΔA÷(ε×d) ×10^9×V_{RT}÷V_{SA}÷T=26.795×ΔA

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
V_{RT}: Total reaction volume, 0.001 L;
V_{SA}: Sample volume, 0.2 mL;
V_E: Extract solution volume, 1 mL;
T: Reaction time, 30 min;
Cpr: Protein concentration, mg/mL;
W: Sample weight, g.
10^9: unit conversion factor, 1mol=10^9 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 min or longer for determination.

Experimental example:

1. Take 0.1084g *Oryza sativa L* leaf and add 1ml Extract solution for homogenate grinding. Take the supernatant and dilute it 2 times. Operate according to the determination steps. Calculate ΔA_T= $A2_T$- $A1_T$= 0.844-0.758 = 0.086, ΔA_B= $A2_B$- $A1_B$= 0.094-0.092 = 0.002, ΔA = ΔA_T - ΔA_B = 0.086-0.002 = 0.084, Enzyme activity calculated by sample mass:
ALDH activity (U/g mass) = 26.795×0.084÷0.1084×2= 41.527 U/g mass

1. Take 0.1023g mouse kidney and add 1ml Extract solution for homogenate grinding. Take the supernatant and dilute it 2 times. Operate according to the determination steps. Calculate ΔA_T= $A2_T$- $A1_T$= 0.875-0.491 = 0.384, ΔA_B= $A2_B$- $A1_B$= 0.094-0.092=0.002, ΔA = ΔA_T - ΔA_B = 0.384-0.002 = 0.382, Enzyme activity calculated by sample mass:
ALDH activity (U/g mass) = 26.795×0.382÷0.1023×2= 200.111 U/g mass

Related Products：

[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)

Related Products:

BC0590/BC0595	Free fatty Acids(FFA) Content Assay Kit
BC2340/BC2345	Lipase(LPS) Activity Assay Kit
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