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/microplate reader

Catalog Number: BC0755

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 10 mL×1	2-8℃
Reagent II	Powder×2	-20℃
Reagent III	Liquid 0.5 mL×1	2-8℃
Reagent IV	Liquid 1 mL×1	2-8℃
Reagent V	Liquid 8 mL×1	2-8℃

Solution Preparation:

Reagent II: Dissolve with 1.5 mL of distilled water one of the bottle before using, and unused liquid can be stored at -20℃ 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 liquid: Before use, the working liquid was prepared according to the sample size in the ratio of Reagent I: Reagent II: Reagent III: Reagent IV =60μL: 20μL: 4μL: 6μL (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 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.

Reagents and Equipment Required but Not Provided:

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

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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. 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/microplate reader 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	-	40
Distilled water	40	-
Working Solution	90	90
Reagent V	70	70

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 1minutes. Put it in a water bath or incubator at 37℃(mammal) or 25℃ (other species) for 30 minutes (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 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:

a. Micro quartz cuvette

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.

$ALDH(U/mg\ prot) = \Delta A \div (\varepsilon \times d) \times 10^9 \times V_{RT} \div (Cpr \times V_{SA}) \div T = 26.795 \times \Delta A \div Cpr$

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 weight.

$ALDH(U/g\ weight) = \Delta A \div (\varepsilon \times d) \times 10^9 \times V_{RT} \div (V_{SA} \times W \div V_E) \div T = 26.795 \times \Delta A \div W$

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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 104 cells or germ.

ALDH(U/10^6 cell)=ΔA÷(ε×d) ×10^9×V_{RT}÷(V_{SA} ÷V_E×N)÷T=26.795×ΔA÷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.

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, 2×10^{-4} L;

V_{SA}: Sample volume, 0.04 mL;

V_E: Extract solution volume, 1 mL;

T: Reaction time, 30 min;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g.

b. 96 well flat-bottom UV 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 (micro quartz cuvette) 0.2 (96-well UV plate), 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 determine.

Experimental example:

1. Take 0.1084g rabbit liver and add 1ml Extract solution for homogenate grinding. Take the supernatant and dilute it 2 times. Operate according to the determination steps. Calculate $ΔA_T$= $A2_T$- $A1_T$= 0.878-0.838 = 0.040, $ΔA_B$= $A2_B$- $A1_B$=0, ΔA = $ΔA_T$ - $ΔA_B$ =0.040-0= 0.040, Enzyme activity calculated by sample mass:

ALDH activity (U/g mass) = 0.040÷(6.22×10^3×0.6)×10^9×2×10^{-4}÷(0.04×0.1023÷1)÷30=16.479U/g mass

2. Take 0.1100g mouse liver and add 1ml Extract solution for homogenate grinding. Take the supernatant and dilute it 2 times. Operate according to the determination steps. Calculate $ΔA_T$=

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$A2_T$-$A1_T$= 1.624-0.798 = 0.826, ΔA_B= $A2_B$- $A1_B$=0, $\Delta A = \Delta A_T - \Delta A_B$ = 0.826-0=0.826, Enzyme activity calculated

by sample mass:

ALDH activity (U/g mass) = $0.826\div(6.22\times10^3\times0.6)\times10^9\times2\times10^{-4}\div(0.04\times0.1023\div1)\div30$=335.343U/g mass

Recent Product Citations：

[1] Fang M, Li Y, Liao Z, Wang G, Cao Q, Li Y, Duan Y, Han Y, Deng X, Wu F, Kamau PM, Lu Q, Lai R. Lipopolysaccharide-binding protein expression is increased by stress and inhibits monoamine synthesis to promote depressive symptoms. Immunity. 2023 Mar 14;56(3):620-634.e11. doi: 10.1016/j.immuni.2023.02.002. Epub 2023 Feb 27. PMID: 36854305.

[2] Zhang Q, Tong J, Zhou W, Zhong Z, Hu Q, Ma Q, Long H, Wu S, Shi X, Ye Q. Antibacterial and antioxidant chitosan nanoparticles improve the preservation effect for donor kidneys in vitro. Carbohydr Polym. 2022 Jul 1;287:119326. doi: 10.1016/j.carbpol.2022.119326. Epub 2022 Mar 8. PMID: 35422292.

[3] Guo L, Li F, Liu H, Kong D, Chen C, Sun S. SIX1 amplification modulates stemness and tumorigenesis in breast cancer. J Transl Med. 2023 Nov 29;21(1):866. doi: 10.1186/s12967-023-04679-2. PMID: 38031089; PMCID: PMC10685563.

[4] Gao Z, Wang D, Zhang H, Yang J, Li M, Lu H, Shen H, Tang Y. An iron-deficient diet prevents alcohol- or diethylnitrosamine-induced acute hepatotoxicity in mice by inhibiting ferroptosis. Curr Res Food Sci. 2022 Nov 5;5:2171-2177. doi: 10.1016/j.crfs.2022.11.001. PMID: 36387594; PMCID: PMC9664348.

[5] Yu PC, Liu D, Han ZX, Liang F, Hao CY, Lei YT, Guo CR, Wang WH, Li XH, Yang XN, Li CZ, Yu Y, Fan YZ. Thymopentin-Mediated Inhibition of Cancer Stem Cell Stemness Enhances the Cytotoxic Effect of Oxaliplatin on Colon Cancer Cells. Front Pharmacol. 2022 Feb 15;13:779715. doi: 10.3389/fphar.2022.779715. PMID: 35242031; PMCID: PMC8886222.

Related Products：

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BC2340/BC2345	Lipase(LPS) Activity Assay Kit
BC1080/BC1085	Alcohol Dehydrogenase(ADH) Activity Assay Kit

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