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Mitochondrial Aldehyde Dehydrogenase-2(ALDH2) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0877-100T-96S

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 15 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 2.8 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Before use, take a bottle of Reagent II and add 1.5 mL of distilled water to dissolve it. Unused reagents can be stored in aliquots at -20°C for 4 weeks, avoiding repeated freezing and thawing;

2. Reagent V: Low boiling point, keep low temperature during use to ensure correct absorption. Seal immediately after use.

3. Working Solution: According to the amount of Reagent I: Reagent II: Reagent III: Reagent IV: Reagent V =110μL: 20μL: 4μL: 6μL: 20μL (160μL, about 1T) mixed for standby, ready for use.

Product Description:

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) belongs to the aldehyde dehydrogenase protein family, which exists in many tissues, especially the liver. This enzyme is mainly involved in the second step of ethanol metabolism process, which oxidizes acetaldehyde into carboxylic acid in mitochondria, and then enters the tricarboxylic acid cycle, which is completely decomposed to remove the toxic effect of acetaldehyde on organisms. In addition, ALDH2 can also participate in the metabolism of nitroglycerin as an esterase, and is an important bioactive agent of nitroglycerin.

ALDH2 catalyzes the conversion of acetaldehyde and NAD^+ to acetic acid and NADH, and the activity of ALDH2 can be calculated by using the change of absorption value of NADH at 340nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, analytical balance, desk centrifuge, water-bath/

constant temperature incubator, adjustable pipette, micro quartz cuvette/96 well flat-bottom UV plate,

mortar /homogenizer/cell ultrasonic crusher, 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. Weigh about 0.1g tissue or collect 5 million cells, add 1mL Extract solution, and homogenize quickly on the ice with a homogenizer or mortar (the homogenizer can grind up and down for about 30 times).
2. Centrifuge 600 g at 4℃ for 10minutes (if higher purity mitochondria are needed, change the centrifugation speed to 1000g).
3. Transfer the supernatant to another centrifuge tube, centrifuge at 4℃ 11000 g for 15minutes, discard the supernatant and leave the precipitation.
4. 400μL Extract solution was added to the precipitation, ultrasonic crushing (200W power, 5 seconds ultrasonic, 10 seconds interval, repeat 15 times), for the determination of ALDH2 activity, if using protein concentration calculation, take 20μL for the determination of protein content.

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℃ for 10 minutes.
3. Operation table:

Reagent Name (μL)	Blank tube (A_B)	Test tube (A_T)
Sample	-	40
Distilled water	40	-
Working Solution	160	160
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. ALDH2 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.

$ALDH2(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(Cpr\times V_{SA})\div T\times F=26.795\times\Delta A\div Cpr\times F$

2) 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.

ALDH2(U/g mass)=ΔA÷(ε×d) ×10^9×V_{RT}÷(V_{SA}×W÷V_E)÷T×F =10.718×ΔA÷W×F

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 10^6 cells or germ.

ALDH2(U/10^6 cell)=ΔA÷(ε×d) ×10^9×V_{RT}÷(V_{SA} ÷V_E×N)÷T×F =10.718×ΔA÷N×F

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, 0.4 mL;

T: Reaction time, 30 minutes;

Cpr: Protein concentration, mg/mL, self determination;

W: Sample mass, g.

N: The total number of cells, 10^6;

10^9: unit conversion factor, 1mol=10^9 nmol;

F: Dilution ratio。

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. Reagent IV is toxic, during the experiment, please wear good protective equipment.
2. Since the extract contains a certain concentration of protein (about 1mg/mL), it is necessary to subtract the protein content of the extract itself (measured separately) when measuring the protein concentration of the sample.
3. When the sample ΔA > 1, it is recommended to dilute the sample with Extraction solution before testing. When ΔA < 0.01, the reaction time can be extended (60min or longer) to determine. Change the formula synchronously during calculation.

Experimental example:

1. 1. Take 0.1067g mouse liver was processed, diluted 4 times with the extract. Operate according to the determination steps. Calculated with 96 well flat-bottom UV plate ΔA_T= $A2_T$- $A1_T$= 0.743-0.169 = 0.574, ΔA_B=$A2_B$-$A1_B$=0.102-0.102=0, ΔA =ΔA_T - ΔA_B = 0.574-0 = 0.574, Enzyme activity calculated by sample mass:

   ALDH2 activity (U/g mass) = 0.574÷(6.22×10^3×0.6)×10^9×2×10^{-4}÷(0.04×0.1067÷0.4)÷30×4

=384.388 U/g mass.

2. Take 0.1069g *Ziziphus jujuba Mill. cv. Dongzao* pulp was processed. Operate according to the determination steps. Calculated with 1mL quartz cuvette $\Delta A_T = A2_T - A1_T = 0.177-0.119 = 0.058$, $\Delta A_B = A2_B - A1_B = 0.102-0.102=0$, $\Delta A = \Delta A_T - \Delta A_B = 0.058-0 = 0.058$, Enzyme activity calculated by sample mass:

   ALDH2 activity (U/g mass) $=0.058\div(6.22\times10^3\times0.6)\times10^9\times2\times10^{-4}\div(0.04\times0.1069\div0.4)\div30$

   =9.692 U/g mass.

3. 8×10^6 cells were collected for sample processing. Operate according to the determination steps. Calculated with 1mL quartz cuvette $\Delta A_T = A2_T - A1_T = 0.160-0.109 = 0.051$, $\Delta A_B = A2_B - A1_B = 0.102-0.102=0$, $\Delta A = \Delta A_T - \Delta A_B = 0.051-0 = 0.051$, Enzyme activity calculated by sample mass:

   ALDH2 activity(U/10^6 cell) $= 0.051\div(6.22\times10^3\times0.6)\times10^9\times2\times10^{-4}\div(0.04\times8\div0.4)\div30=0.114$ U/10^6 cell.