Alcohol Dehydrogenase (ADH) Activity Assay Kit

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

Detection equipment: Spectrophotometer/Microplate reader

Cat No:NA0549

Size:100T/96S

Components

Extract solution: 100 mL×1, store at 4℃.Contains insoluble substances, shake well before use;

Reagent I:20 mL×1, store at 4℃. Transfer Reagent II to Reagent I when the solution will be used, split charging and store at -20℃.

Reagent II:Powder×1, store at -20℃.

Reagent III:2 mL×1, store at 4℃.

Description

Alcohol dehydrogenase (ADH) is a key enzyme in the metabolism of short chain alcohols. It catalyzes the reversible conversion of ethanol and acetaldehyde, and plays an important role in many physiological processes. In mammals, ADH is mainly produced in the liver. Liver damage causes ADH to be released into serum. The activity of serum ADH reflects whether the liver function is abnormal.

ADH catalyzes the reduction of acetaldehyde by NADH to ethanol and NAD^+. NADH has an absorption peak at 340 nm but NAD^+ not, the activity of ADH is calculated by measuring the rate of absorbance decline at 340 nm.

Required but not provided

Mortar/homogenizer, ice, low temperature centrifuge,spectrophotometer/microplate reader, micro quartz cuvette/96 well flat-bottom plate (UV plate), adjustable pipetteand distilled water.

Protocol

I. Crude enzyme extraction:

1. Tissue:

The mass of tissue (g): the volume of Extract solution(mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extract solution) for ice bath homogenate. Centrifuge at 16000 ×g for 20 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Bacteria and fungi: the number of cells (10^4): the volume of Extract solution(mL) is 500~1000:1 (1 mL of Extract solution is recommended to be added to 5 million cells), the cells are broken by ultrasonic wave in ice bath (Power: 300W, ultrasonic wave: 3s, interval: 7s, total time: 3 minutes). Centrifuge at 16000 ×g for 20 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum and other Liquids:

Direct determination.

II. Procedure

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

2. Keep the ReagentI in a 25℃ water bath for more than 30 minutes.

3. Blank tube:

Add 20 μL of distilled water, 160 μL of Reagent I and 20 μL of Reagent III to the micro quartz cuvette/96 well flat-bottom plate (UV plate) in turn. Mix them quickly and measure the change of absorption value at 340 nm, record the absorption value at 15 s and 75 s respectively, record them as A1 and A2. ΔA_B = A1-A2. Only one or two blank tubes need to be test.

4. Test tube:

Add 20 μL of supernatant, 160 μL of Reagent I and 20 μL of Reagent III to the micro quartz cuvette/96 well flat-bottom plate (UV plate) in turn. Mix them quickly and measure the change of absorption value at 340 nm, record the absorption value at 15 s and 75 s respectively, record them as A3 and A4. ΔA_T = A3-A4.

III. Calculation of ADH activity

A. The calculation formula according to the determination of micro quartz cuvette

(1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmolNADH per minute at 25℃everymilligram tissue protein.

ADH (μmol/min/mg prot)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(Cpr\times V_{SV})\div T=1.61\times(\Delta A_T-\Delta A_B)\div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ everygram tissue.

ADH (μmol/g fresh weight) = $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times W)\div T$

$=1.61\times(\Delta A_T-\Delta A_B)\div W$

(3) Cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every 10 thousand cells.

ADH (μmol/10^4 cell) = $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times N)\div T$

$=1.61\times(\Delta A_T-\Delta A_B)\div N$

(4) Liquids

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every milliliter liquid.

ADH (μmol/mL liquids) = $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div V_{SV}\div T$

$=1.61\times(\Delta A_T-\Delta A_B)$

ε: The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

d: Cuvette light diameter, 1 cm;

V_{RV}: The total volume of reaction system, 200 μL=2×10^{-4}L;

10^6: 1 mol=1×10^6 μmol;

V_{SV}: The volume of sample, 20 μL=0.02 mL;

V_{STV}: The volume of extract solution, 1 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: The number of cells.

B. The calculation formula according to the determination of 96 well plate:

(1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every milligram tissue protein.

ADH (μmol/min/mg prot)=$[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(Cpr\times V_{SV})\div T$

$=2.68\times(\Delta A_T-\Delta A_B)\div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every gram tissue.

ADH (μmol/g fresh weight)＝$[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times W)\div T$

$=2.68\times(\Delta A_T-\Delta A_B)\div W$

(3) Cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every 10 thousand cells.

ADH (μmol/10^4 cell) = $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times N)\div T$

$=2.68\times(\Delta A_T-\Delta A_B)\div N$

(4) Liquids

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 25℃ every milliliter liquid.

ADH (μmol/mL liquit) = $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div V_{SV}\div T$

$=2.68\times(\Delta A_T-\Delta A_B)$

ε: The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

d: Cuvette light diameter, 0.6 cm;

V_{RV}: The total volume of reaction system, 200 μL=2×10^{-4}L;

10^6: 1 mol=1×10^6 μmol;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

V_{SV}: The volume of sample, 20 μL=0.02 mL;

V_{STV}: The volume of extract solution, 1 mL;

T: Reaction time, 1 minute;

N: The number of cells.

Note:

The protein concentration of supernatant needs to be determined separately. It is recommended to use BCA

protein content determination kit of our company.

Experimental instances：

1. Take 0.1g of rat liver, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. CalculateΔA_B=A1-A2=0.5718-0.5648=0.007， ΔA_T=A3-A4=0.8351-0.5341=0.301, calculate the enzyme activity according to sample weight:

ADH (U/g weight) =1.61×(ΔA_T–ΔA_B)÷W=1.61×(0.301-0.007)÷0.1=4.7334 U/g weight.

2. Take serum of horse to detect directly, calculateΔA_B=A1-A2=0.5718-0.5648=0.007， ΔA_T=A3-A4=0.6369-0.6036=0.0333, calculate the enzyme activity according to volume of serum:

ADH ((U/mL) =1.61×(ΔA_T–ΔA_B)=1.61×(0.0333-0.007)=0.042343 U/mL.

3. Take serum of mouse to detect directly, calculateΔA_B=A1-A2=0.5718-0.5648=0.007， ΔA_T=A3-A4=0.7381-0.7093=0.0288, calculate the enzyme activity according to volume of serum:

ADH (U/mL) =1.61×(ΔA_T–ΔA_B)=1.61×(0.0288-0.007)=0.035098 U/mL.

Related products：

NA0822/NA0580	Free fatty Acids(FFA) Assay Kit
NA0701/NA0460	Lipase(LPS) Activity Assay Kit
NA0842/NA0600	Plant Lipoxygenase (LOX) Assay Kit
NA0808/NA0566	Aldehyde Dehydrogenase (ALDH)