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# Alcohol Dehydrogenase (ADH) Activity Assay Kit

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

**Detection equipment:**Spectrophotometer

**Cat No:**AK0262

**Size:**50T/48S

## Components

Extract: 50 mL×1, store at 4℃;

Reagent I:40 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:5 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 a absorption peak at 340 nm but $NAD^+$ not, the activity of ADH can calculated by measuring the rate of absorbance decline at 340 nm.

## Required but not provided

Mortar/homogenizer, ice, low temperature centrifuge,spectrophotometer, 1 mL quartz cuvette, 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 for 20 minutes at 16000 ×g 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: 3 s, interval: 7 s, total time: 3 minutes). Centrifuge at 16000 ×g for 20 minutes at 4℃, the supernatant is taken and placed on ice for testing.

3. Serum and other liquids: direct determination.

### II. Procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Keep the Reagent I in a 25℃ water bath for more than 30 minutes.
3. Blank tube:

Add 100 μL of distilled water, 800 μL of Reagent I and 100 μL of Reagent III to the 1 mLquartz cuvette 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. $\Delta A_B$ = A1-A2. The blank tube only need to be test one or two tubes.

4. Measuring tube:

Add 100 μL of supernatant, 800 μL of Reagent I and 100 μL of Reagent III to the 1 mLquartz cuvette 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. $\Delta A_T$= A3-A4.

**III. Calculation of ADH activity**

(1) Protein concentration

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

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

(2) Sample weight

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

$$ADH\ (\mu mol/g\ fresh\ weight) = [(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times W)\div T$$
$$=1.61\times(\Delta A_T-\Delta A_B)\div W$$

(3) Cells

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

$$ADH\ (\mu mol/10^4\ cell) = [(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_{SV}\div V_{STV}\times N)\div T$$
$$=1.61\times(\Delta A_T-\Delta A_B)\div N$$

(4) Liquids

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

$$ADH\ (\mu mol/mL\ liquids) = [(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div V_{SV}\div T$$
$$=1.61\times(\Delta A_T-\Delta A_B)$$

ε: The molar extinction coefficient of NADH, $6.22\times10^3$ L/mol/cm;

d: Cuvette light diameter, 1 cm;

$V_{RV}$ : The total volume of reaction system, 1000 μL=$1\times10^{-3}$L;

$10^6$ : 1 mol=$1\times10^6$ μmol;

$V_{SV}$ : The volume of supernatant, 100 μL=0. 1 mL;

$V_{STV}$ : The volume of Extract, 1 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g.
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. Calculate$\Delta A_B$=A1-A2=0.558-0.557=0.001， $\Delta A_T$=A3-A4=0.812-0.587=0.225, calculate the enzyme activity according to sample weight:
ADH (U/g weight) =1.61×($\Delta A_T$–$\Delta A_B$)÷W=1.61×(0.225–0.001)÷0. 1=3.6064U/g weight.
2. Take serum of mouse to detect directly, calculate$\Delta A_B$=A1-A2=0.558-0.557=0.001，$\Delta A_T$=A3-A4=0.708-0.676=0.032, calculate the enzyme activity according to volume of serum:
ADH (U/mL) =1.61×($\Delta A_T$–$\Delta A_B$)=1.61×(0.032–0.001)=0.04991 U/mL.

**Related products：**

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|---|---|
| AK0536/AK0535 | Free fatty Acids(FFA) Assay Kit |
| AK0384/AK0383 | Lipase(LPS) Activity Assay Kit |
| AK0297/AK0296 | Plant Lipoxygenase (LOX) Assay Kit |
| AK0269/AK0268 | Aldehyde Dehydrogenase (ALDH) |