(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Alcohol dehydrogenase Assay Kit (Tissue)

Catalog No.: BC00202

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Alcohol dehydrogenase Assay Kit (Tissue)
Detection Method	Colorimetric
Sample Type	Tissue
Assay Type	Quantitative
Detection Instrument	Ultraviolet spectrophotometer (340 nm)

Product Introduction

Alcohol dehydrogenase (ADH) is a type of dehydrogenase widely present in many organisms, which catalyzes the interconversion between alcohols and aldehydes or ketones. In humans and many other animals, it functions to degrade toxic alcohols. For instance, ADH oxidizes methanol to formaldehyde, which is eventually converted into formic acid. It also participates in the formation of some aldehyde, ketone and alcohol groups during the biosynthesis of various metabolites.

Principle

Based on the principle that alcohol dehydrogenase (ADH) catalyzes the reaction of oxidized coenzyme I, the enzyme activity is determined by measuring the change rate of absorbance at 340 nm.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	20 mL	Store at 4°C, dilute 1:1 with double distilled water immediately before use to prepare the working solution.
Reagent 2	Liquid	1 bottle	Store at 4°C.

Reagent 3	Powder	4 vials	Store below -20°C. Before use, dissolve each vial of powder with 10 mL of double distilled water to prepare the working solution. The prepared solution can be stored at -20°C for about one week.

Storage

The validity period of the kit is 3 months.

Preparation

Ultraviolet spectrophotometer with adjustable wavelength of 340 nm, 0.5 cm optical path quartz cuvette, vortex mixer, centrifuge, 37°C water bath, stopwatch, distilled water, normal saline, and protein assay reagents (available from our company).

- **Sample handling**

  Preparation of 10% Tissue Homogenate: Accurately weigh the tissue sample. Add 9 times the volume of normal saline at a mass (g) to volume (mL) ratio of 1:9. Perform mechanical homogenization under ice-water bath conditions. Centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for determination.

Operation process

	Blank tube	**Assay tube**
Reagent 1 working solution (mL)	0.65	0.65
Reagent 2 (mL)	0.05	0.05
Reagent 3 working solution (mL)	0.75	0.75
Mix well and pre-incubate at 37°C for 10 minutes.		
Supernatant (mL)		0.05

Double distilled water (mL)	0.05	
Start timing immediately while mixing the sample with the reagent and mix thoroughly at once. At 15 seconds, measure the OD value A1 at 340 nm with a 0.5 cm optical path. Quickly place the reaction solution in a 37°C water bath, then determine the OD value A2 at 10 minutes and 15 seconds. Calculate △A = A2 − A1.		

Calculation

Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol product per minute per milligram of tissue protein at 37°C.

$$\text{ADH activity (U/mg)} = \frac{\Delta A_{\mathbf{Assay}} - \Delta A_{\mathbf{Blank}}}{6.22 \times d} \times \frac{V_{Total}}{V_{Sample}} \div T \times 1000 \div Cpr$$

Annotation:

V_{Total}: Total volume of reaction system, 1.5 mL

V_{Sample}: Volume of sample added, 0.05 mL

T: Reaction time, 10 min

Cpr: Protein concentration of homogenate, mg/mL

d: Cuvette optical path, 0.5 cm

6.22: Micromolar extinction coefficient of the substrate

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Under normal conditions, the ADH content in the sample is relatively low, so no obvious change

in OD value may be observed during the determination, which is regarded as a normal phenomenon.

4. The spectrophotometer shall be zeroed with distilled water before each OD measurement to reduce instrumental error.
5. Repeated freeze-thawing of samples and delayed detection after homogenization will also affect (reduce) the enzyme activity.