



CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit

Cat #: KTB3030

Size: 48 T/48 S

96 T/96 S

	Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit		
REF	Cat #: KTB3030	LOT	Lot #: Refer to product label
	Applicable samples: Animal Tissue, Bacteria, Serum, Plasma		
	Storage: Stored at -20°C for 6 months, protected from light		

Assay Principle

Alcohol dehydrogenase (ADH) is a key enzyme in the metabolism of short-chain alcohols in organisms, which catalyzes the reversible conversion of ethanol to acetaldehyde and plays an important role in many physiological processes. Mammalian ADH is mainly produced in the liver, and liver injury leads to the release of ADH into the serum. The activity of serum ADH reflects whether the liver function is abnormal or not. CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit provides a simple, convenient and rapid ADH activity detection method, which is suitable for the detection of animal tissues, bacteria, serum (plasma) and other samples. The detection principle is that ADH catalyzes NADH to reduce acetaldehyde to ethanol and NAD⁺, NADH has a characteristic absorption peak at 340 nm, but NAD⁺ does not. Determine the decline rate of 340 nm absorbance and calculate the activity of ADH.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	60 mL	60 mLx2	4°C
Reagent II	9.9 mL	19.8 mL	4°C
Reagent III	Powderx1 vial	Powderx1 vial	-20°C, protected from light
Reagent IV	1.2 mL	2.4 mL	4°C, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, water bath
- Homogenizer (for tissue samples)

Reagent Preparation

Reagent I : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. The reagent contains insoluble matter, shake well use it and should be centrifuged when used in the control sample.

Working Solution: Before use, Reagent II poured into Reagent III, fully mixed and ready for use. Unused reagents can also be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Note: Reagent I or Reagent IV is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Sample Preparation

Note: Fresh samples are recommended. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 16,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Bacteria: Collect 5×10^6 bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 16,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Serum (Plasma): Direct detection.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Working Solution for 30 min at room temperature (25°C).

3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	20
Reagent I	20	0
Reagent IV	20	20
Working Solution	160	160

4. After mixing quickly, record the absorbance values of 20 s and 1 min 20 s at 340 nm, mark as A_1 and A_2 , and calculate $\Delta A_{\text{Test}} = (A_{\text{Test}1} - A_{\text{Test}2}) - (A_{\text{Blank}1} - A_{\text{Blank}2})$.

Note: Blank well only needs to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Reagent I, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculation of ADH activity in serum

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum per min.

$$\text{ADH (U/mL)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9] \div V_{\text{Sample}} \div T = 3,215 \times \Delta A_{\text{Test}}$$

2. Calculation of ADH activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

$$\text{ADH (U/mg prot)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{Sample}} \times \text{Cpr}) \div T = 3,215 \times \Delta A_{\text{Test}} \div \text{Cpr}$$

(2) Calculation according to the weight of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min.

$$\text{ADH (U/g fresh weight)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9] \div (W \div V_{\text{Reagent I}} \times V_{\text{Sample}}) \div T = 3,215 \times \Delta A_{\text{Test}} \div W$$

3. Calculation of ADH activity in bacteria

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 bacteria per min.

$$\text{ADH (U/10}^4\text{)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{Sample}} \div V_{\text{Reagent I}} \times 500) \div T = 6.43 \times \Delta A_{\text{Test}}$$

Where: V_{Total} : the total volume of the reaction system, $0.2 \text{ mL} = 2 \times 10^{-4} \text{ L}$; $V_{\text{Reagent I}}$: the volume of the Reagent I, 1 mL; V_{Sample} : the volume of the supernatant in the reaction system, 0.02 mL; ϵ : NADH molar extinction coefficient, $6.22 \times 10^3 \text{ L/mol/cm}$; d : 96-well UV plate diameter, 0.5 cm; Cpr : protein concentration (mg/mL); T : reaction time, 1 min; W : sample weight, g; 500: total number of bacteria, 5 million; 10^9 : unit conversion factor, $1 \text{ mol} = 10^9 \text{ nmol}$.

B. Microquartz cuvette calculation formula

The optical diameter d : 0.5 cm in the above calculation formula can be adjusted to d : 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB3020	CheKine™ Micro NADP-Malate Dehydrogenase (NADP-MDH) Activity Assay Kit
KTB3021	CheKine™ Micro NAD-Malate Dehydrogenase (NAD-MDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.