EZMeta™ Alcohol Dehydrogenase (ADH) Colorimetric Activity Kit

Cat #: D-AKC3030

Size: 48T / 96T

Storage: Stored at -20℃ for 6 months, protected from light

Product Information

Applicable samples: Animal Tissue, Bacteria, Serum, Plasma

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. EZMeta™ Alcohol Dehydrogenase (ADH) Colorimetric Activity 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	50 mL	100 mL	4°C
Reagent Ⅱ	9 mL	18 mL	4°C
Reagent Ⅲ	1	1	-20°C, protected from light
Reagent Ⅳ	1 mL	2 mL	4°C, protected from light

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 ReagentⅢ, 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.

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 EZMeta™ Alcohol Dehydrogenase (ADH) Colorimetric Activity Kit, 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
ReagentⅣ	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 $\Delta A_{Test}=(A_{Test2}-A_{Test1})-(A_{Blank2}-A_{Blank1})$.

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 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.

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

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.

ADH (U/mg prot)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T=**3,215×ΔA_{Test}÷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.

ADH (U/g fresh weight)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(W÷$V_{Reagent\ I}$ ×V_{Sample})÷T=**3,215×ΔA_{Test}÷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.

ADH (U/10^4)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Reagent\ I}$ ×500)÷T=**6.43×ΔA_{Test}**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; $V_{Reagent\ I}$: 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, 6.22×10^3 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 mol=10^9 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.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.