CheKine™ Micro Ethanol Content Detection Kit

Cat #: KTB3031 Size: 48 T/48 S 96 T/96 S

	Micro Ethanol Content Detection Kit		
REF	**Cat #:** KTB3031	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma or other Liquids		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Alcohol is the general name of alcoholic (ethanol) beverages, ethanol is the main component of alcohol, is one of the important indicators to measure the quality of wine. Ethanol can be used to manufacture acetic acid, beverages, flavors, dyes, fuels, etc. In medical treatment, ethanol with a volume fraction of 70% to 75% is commonly used as a disinfectant. Ethanol is widely used in chemical industry, medical care, food industry, agricultural production and other fields. CheKine™ Micro Ethanol Content Detection Kit can detect biological samples such as Animal and Plant Tissues, Cells, Bacteria, Serum or Plasma. In this kit, ethanol is oxidized and dehydrogenated to produce acetaldehyde under the catalysis of ethanol dehydrogenase, while NAD is reduced to NADH, which makes WST-8 orange color under the action of 1-mPMS. Ethanol content can be measured by the change of absorbance value at 450 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	6 mL	12 mL	4℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	5 mL	10 mL	4℃
Reagent Ⅳ	0.75 mL	1.5 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, 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 visible light spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microquartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, centrifuge

• Deionized water
• Homogenizer (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅱ: Prepare before use, 48 T add 3 mL Reagent Ⅲ, 96 T add 6 mL Reagent Ⅲ to dissolve it for use. The unused Reagent Ⅱ can be stored stored at -20℃ for 1 month after packaging, avoid repeated freeze-thaw cycles.
Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Note: Reagent Ⅳ has certain irritation, so personal protection is recommended during use.
Working Reagent: Prepare 160 μL of Working Reagent for each well before use. Add 100 μL of Reagent Ⅰ, 50 μL of Reagent Ⅱ, and 10 μL Reagent Ⅳ, mix well.
Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
5 μmol/mL Standard: Prepare before use, add 58.4 μL Standard to 941.6 μL deionized water to prepare 1,000 μmol/L Standard. Add 50 μL 1,000 μmol/L Standard to 950 μL deionized water to prepare 50 μmol/L Standard. Then add 100 μL 50 μmol/L Standard to 900 μL deionized water to prepare 5 μmol/L Standard, the 5 μmol/L Standard is used for the detection of the following standard well.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.
1. Tissues: Weigh 0.1 g tissue , add 1 mL deionized water, homogenize on ice. Centrifuge at 8,000 g for 10 min at room temperature. Take the supernatant for testing.
2. Cells and Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation, add 1 mL deionized water, ultrasonically disrupt cells 3 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, total time for 3 min). Then centrifuge at 8,000 g for 10 min at room temperature. Take the supernatant for testing.
3. Serum, Plasma and other Liquid Samples: Direct detection.
Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible light spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible light spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well plate or microquartz cuvette)

Regent	Standard well (μL)	Test well (μL)
Sample	0	40
Standard	40	0
Working Reagent	160	160

3. Mix well, record the absorbance values of 0 min and 10 min at 450 nm, mark as A_1 and A_2, the standard well is marked as $A_{standard}$, and the test well is marked as A_{Test}, and calculate $\Delta A=A_2-A_1$.
Note: Standard well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} is less than 0.001, the sample size can be appropriately increased. If the ΔA_{Test} is greater than 0.6, the sample can be appropriately diluted with deionized water or reduce the sample quality

used for extraction, the calculated result multiplied by the dilution factor.

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.

Calculation of ethanol content

(1) Calculated by protein concentration:

Ethanol (μmol/mg prot)=$[C_{Standard}\times(\Delta A_{Test}\div\Delta A_{Standard})\times V_{Sample}]\div(Cpr\times V_{Sample}\div V_{Sample\ Total})$=$\mathbf{5\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculation according to the weight of the sample:

Ethanol (μmol/g fresh weight)=$[C_{Standard}\times(\Delta A_{Test}\div\Delta A_{Standard})\times V_{Sample}]\div(W\times V_{Sample}\div V_{Sample\ Total})$=$\mathbf{5\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculation according to cell number

Ethanol (μmo/10^4)=$[C_{Standard}\times(\Delta A_{Test}\div\Delta A_{Standard})\times V_{Sample}]\div(n\times V_{Sample}\div V_{Sample\ Total})$=$\mathbf{5\times\Delta A_{Test}\div\Delta A_{Standard}\div n}$

(4) Calculation according to the volume of liquid

Ethanol (U/mL)=(μmol/mL)=$C_{Standard}\times(\Delta A_{Test}\div\Delta A_{Standard})$=$\mathbf{5\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: The concentration of standard, 5 μmol/mL; V_{Sample}: The volume of the sample in the reaction system, 0.025 mL; $V_{Sample\ Total}$: The volume of deionized water added, 1 mL; Cpr: protein concentration, mg/mL; n: Total number of cells or bacteria, in tens of thousands; W: sample weight, g.

Typical Data

The following data is for reference only, and experimenters need to test the samples based on their own experiments.

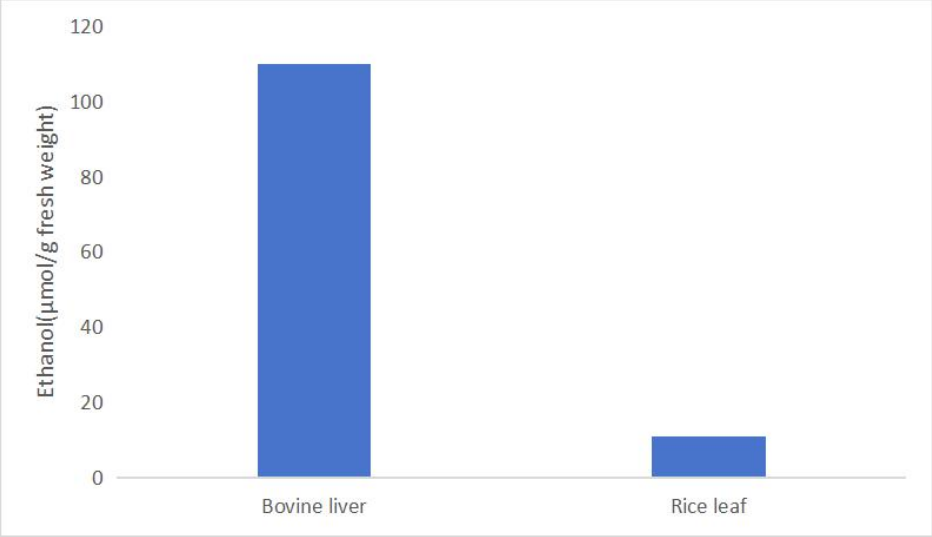

Figure 1. Determination of ethanol content in bovine liver and rice leaf was detected by this kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit
KTB1100	CheKine™ Micro Lactate 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.