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

Catalog No：E-BC-K891-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.27–10.0 μmol/mL

Elabscience®Ethanol Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

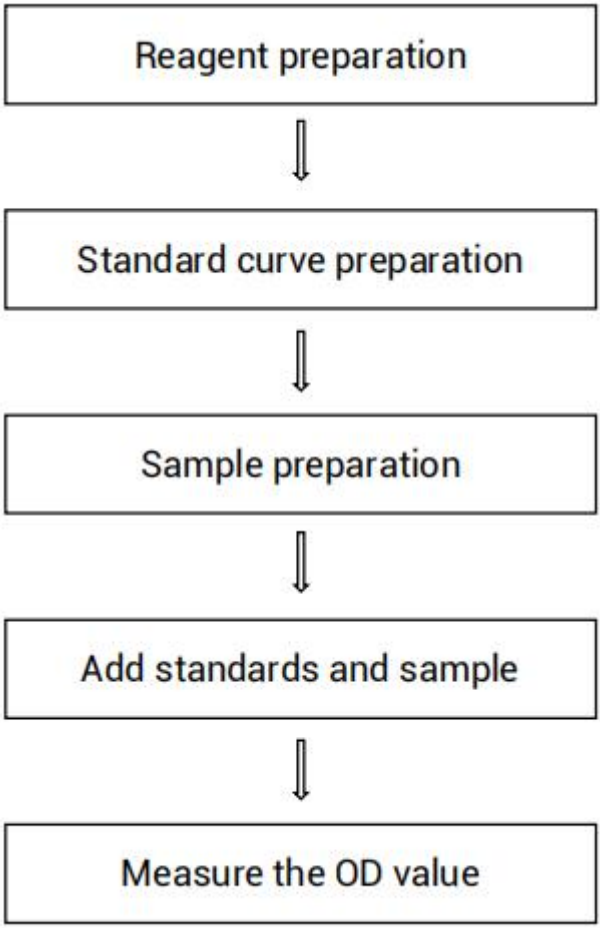
Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure ethanol content in serum (plasma) and wine samples.

Detection principle

Alcohol (ethanol C_2H_5OH) is one of the most widely used beverage, low dose of alcohol may improve blood circulation, and heavy drinking can lead to various diseases. Ethanol content determination in the blood is an important judgment of alcoholism, through detecting alcohol content in blood after intake of alcohol, it is convenient and rapid to monitor and study the metabolic process of ethanol in the body, which can provide the corresponding indexes and basis for the research of preventing and alleviating alcoholism.

Ethanol dehydrogenase can catalyze oxidative dehydrogenation of ethanol to acetaldehyde, and NAD+ is reduced to produce NADH. NADH, under the action of 1-mPMS, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm. Therefore, ethanol content can be quantified by measure the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution A	10 mL × 1 vial	20 mL × 1 vial	-20℃, 12 months
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months shading light
Reagent 3	Buffer Solution B	7 mL × 1 vial	14 mL × 1 vial	-20℃, 12 months
Reagent 4	Substrate	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months shading light
Reagent 5	Chromogenic Agent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20℃, 12 months shading light
Reagent 6	10 μmol/mL Standard Solution	1.8 mL × 1 vial	1.8 mL × 2 vials	-20℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Centrifuge, Micropipettor, Vortex mixer, Incubator (37℃)

Reagents:

Double distilled water

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② Preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 180 μL of double distilled water, mix well to dissolve. The prepared solution should be stood at room temperature for 30 min protected from light before use. Store at 2-8°C for 4 days protected from light.

③ Preparation of substrate working solution：
Dissolve one vial of substrate with 170 μL of double distilled water, mix well to dissolve. Store at -20°C for 4 days protected from light.

④ Preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 256 μL of reaction working solution (mix well 160 μL of buffer solution A, 2 μL of enzyme working solution, 37 μL of buffer solution B, 2 μL of substrate working solution and 8 μL of chromogenic agent). Keep the prepared solution on ice protected from light during use. The prepared solution should be prepared on spot and used up within 0.5 hours. (Preparation of the reaction working solution after the standard and sample are added to the wells)

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 μmol/mL standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 10, 9, 8, 6, 4, 3, 2, 0 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**2**	**3**	**4**	**6**	**8**	**9**	**10**
10 μmol/mL standard (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Beer (2.8% alcohol)	60-100
White wine (12% alcohol)	250-300

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Avoid bubbles when adding reaction working solution. Break the bubbles before measurement if there are some bubbles.

② After adding the reaction working solution, the microplate should be with shading light.

③ Dissolve enzyme reagent with 180μL of double distilled water. The prepared solution should be stood at room temperature for 30 min before use.

④ Preparation of the reaction working solution after the standard and sample are added to the wells.

Operating steps

① Standard well: Take 40 μL of standard with different concentrations to corresponding wells.
Sample well: Take 40 μL of sample to sample wells.

② Add 160 μL of reaction working solution into each well.

③ Mix fully with microplate reader for 3 s. Measure the OD value of each well at 450 nm with microplate reader, recorded as A_1 (complete within 2 min).

④ Incubate at 37℃ with shading light for 10 min.

⑤ Measure the OD value of each well at 450 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

$$\text{Ethanol content (μmol/mL)} = (\Delta A_{450} - b) \div a \times f$$

[Note]

ΔA_{450}: $\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}$ (ΔA_{Blank} is the change OD value when the standard concentration is 0).

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three beer samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/mL)	0.80	2.60	6.80
%CV	3.8	3.5	3.2

Inter-assay Precision

Three beer samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/mL)	0.80	2.60	6.80
%CV	4.8	5.1	5.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/mL)	2.7	5	7.5
Observed Conc. (μmol/mL)	2.6	4.7	7.2
Recovery rate (%)	98	94	96

Sensitivity

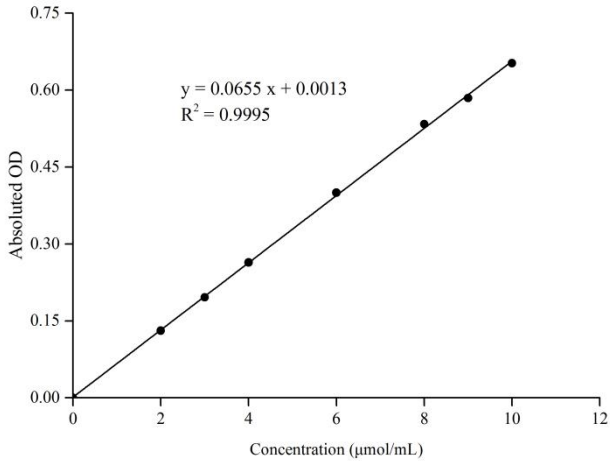
The analytical sensitivity of the assay is 0.27 μmol/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/mL)	**0**	**2**	**3**	**4**	**6**	**8**	**9**	**10**
Average OD	0.016	0.147	0.212	0.280	0.416	0.550	0.601	0.669
Absoluted OD	0.000	0.131	0.196	0.264	0.400	0.534	0.585	0.653



Appendix Π Example Analysis

Example analysis：

For beer (marked with alcohol content ≥2.8%, ≥476 μmol/mL), take 40 μL of beer sample diluted for 100 times and carry the assay according to the operation table.The results are as follows:

Standard curve: y = 0.0655 x + 0.0013, the average OD value of the blank (A_1) is 0.05, the average OD value of blank (A_2) is 0.066, the average OD value of ΔA Blank is 0.016, the average OD value of the sample (A_1) is 0.065, the average OD value of sample (A_2) is 0.434, the average OD value of ΔA sample is 0.369, and the calculation result is:

Ethanol content (μmol/mL)=（0.369-0.016-0.0013）÷0.0655×100=536.95 μmol/mL

Appendix III Publications

1. Shen H, Zhou L, Zhang H, et al. Dietary fiber alleviates alcoholic liver injury via Bacteroides acidifaciens and subsequent ammonia detoxification[J]. Cell Host & Microbe, 2024, 32(8):23. DOI: 10.1016/j.chom.2024.06.008.
2. Chen Y, Yu M, Liu M, et al. A Solvent Exchange Induced Robust Wet Adhesive Hydrogels to Treat Solid Tumor Through Synchronous Ethanol Ablation and Chemotherapy[J]. Advanced Science, 2024, 11(24). DOI:10.1002/advs.202309760.
3. Cheng S, Xueheng C, Yong C, et al. Alpha-lipoic Acid Protects Against Chronic Alcohol Consumption-induced Cardiac Damage by the Aldehyde Dehydrogenase 2–associated PINK/Parkin Pathway[J]. Journal of cardiovascular pharmacology. 2023(5): 82. DOI: 10.1097/FJC.0000000000001480.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.