Technical Manual

Ethanol Colorimetric Assay Kit

- **Catalogue Code: MAES0203**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.27–17.0 μmol/mL

Sensitivity:

0.27 μmol/mL

Storage:

-20°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. Background:

Alcohol (ethanol C2H5OH) 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.

3. Intended Use:

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

4. Detection Principle:

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.

5. Kit Components & Storage:

Item	Specification	Storage
Buffer Solution A	20 mL × 1 vial	-20°C, 3 months
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 3 months
Buffer Solution B	14 mL × 1 vial	-20°C, 3 months
Chromogenic Agent	1.5 mL × 2 vials	-20°C, 3 months, avoid direct sunlight
Standard Solution (17 μmol/mL)	1.8 mL × 2 vials	-20°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Incubator
- Centrifuge
- Microplate Reader (440-460 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)

6. Assay Notes:

1. Prevent 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 avoided with direct sunlight.

7. Reagent Preparation:

1. Bring enzyme reagent on the ice box and the other reagents to room temperature before use.

2. Preparation of **enzyme working solution:** Dissolve enzyme reagent with 180 μL of double distilled water and mix fully, the prepared solution should be used in 12 h. Mix the enzyme reagent solution and buffer solution B at the ratio of 1:17 fully. Place it on the ice box and prepare the fresh needed amount before use. The prepared solution should be used within 0.5 h.

3. Preparation of **reaction working solution:** Mix the buffer solution A, enzyme working solution and chromogenic agent at the ratio of 10: 5: 1. Prepare the fresh needed amount by avoiding direct sunlight before use and prepared solution should be used in 0.5 h.

8. Sample Preparation:

1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:

Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.27–17.0 μmol/mL).

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

Sample Type:	Dilution Factor:
Beer (3.6% alcohol)	40-80
White wine (12% alcohol)	250-300

Note: The diluent is double distilled water.

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

Dilute 17 μmol/mL standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 17, 15.3, 13.6, 10.2, 6.8, 5.1, 3.4, 0 μmol/mL.

The measurement of samples

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°C and avoiding direct sunlight for 10 min.

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

Operation Table

	Standard well	Sample well
Standard of different concentrations (μL)	40	
Sample (μL)		40
Reaction working solution (μL)	160	160
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.		
Incubate at 37°C and avoid direct sunlight for 10 min.		
Measure the OD value of each well at 450 nm with microplate reader, recorded as A_2. $\triangle A = A_2 - A_1$.		

11. Calculations:

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Liquid samples:

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

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

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

f: Dilution factor of sample before tested.

12. Performance Characteristics:

Detection Range	0.27–17.0 μmol/mL
Sensitivity	0.27 μmol/mL
Average recovery rate (%)	96
Average inter-assay CV (%)	5
Average intra-assay CV (%)	3.5

Analysis

Take 40 μL of beer sample diluted for 50 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0194 x + 0.0057, the average OD value of the blank (A_1) is 0.105, the average OD value of blank (A_2) is 0.121, the average OD value of ΔA_{Blank} is 0.016, the average OD value of the sample (A_1) is 0.170, the average OD value of sample (A_2) is 0.497, the average OD value of ΔA_{sample} is 0.327, and the calculation result is:

$$\textbf{Ethanol content (μmol/mL)} = \textbf{(0.327-0.016-0.0057)} \div \textbf{0.0194} \times \textbf{50}$$

$$= \textbf{786.86 μmol/mL}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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