TECHNICAL MANUAL

Ethanol Colorimetric Assay Kit

- **SKU CODE:** MAES0203
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure OD values

2. Intended use

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

3. Detection principle

Alcohol (ethanol C_{2H}_5OH) is one of the most widely used beverages. Low doses of alcohol may improve blood circulation, while heavy drinking can lead to various diseases. Ethanol content determination in blood is an important indicator for diagnosing alcoholism. By detecting alcohol content in blood after intake, it provides a convenient and rapid method to monitor and study the metabolic process of ethanol in the body, which can provide corresponding indices and basis for research on preventing and alleviating alcoholism.

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

4. 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°C, 12 months
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 3	Buffer Solution B	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
Reagent 4	Substrate	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 5	Chromogenic Agent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 6	10 μmol/mL Standard Solution	1.8 mL × 1 vial	1.8 mL × 2 vials	-20°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

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 small volume reagents, please centrifuge before use to obtain sufficient amounts of reagents.

6. 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 kept 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, prepare sufficient reaction working solution according to the test wells. For example, prepare 256 μL of reaction working solution by mixing 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. Mix well. Keep the prepared solution on ice protected from light during use. The prepared solution should be prepared fresh and used within 0.5 hours. (Prepare 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 serial concentrations. The recommended dilution gradient is as follows: 10, 9, 8, 6, 4, 3, 2, 0 µmol/mL.

7. Standard curve preparation

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

8. Sample preparation

Sample preparation:

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

Dilution of sample:

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

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 perform a pretest to confirm the dilution factor.

9. The key points of the assay

1. Avoid bubbles when adding reaction working solution. Break the bubbles before measurement if there are any bubbles.
2. After adding the reaction working solution, the microplate should be protected from light.
3. Dissolve enzyme reagent with 180 μL of double distilled water. The prepared solution should be kept at room temperature for 30 min before use.
4. Prepare the reaction working solution after the standard and sample are added to the wells.

10. 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°C protected from light for 10 min.
5. Measure the OD value of each well at 450 nm with microplate reader, recorded as A_2. ΔA = A_2 - A_1.

11. 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 absolute OD value.

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

The sample:

Ethanol content (μmol/mL) = (ΔA_450 - b) ÷ a × f

[Note]

ΔA_450: ΔA_Sample - ΔA_Blank (ΔA_Blank is the change OD value when the standard concentration is 0).

f: Dilution factor of sample before tested.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel replicates of each concentration to obtain an average recovery rate of 96%.

Recovery

Standard	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.

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), the standard curve and data are provided below for reference only:

Standard curve data

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
Absolute OD	0.000	0.131	0.196	0.264	0.400	0.534	0.585	0.653

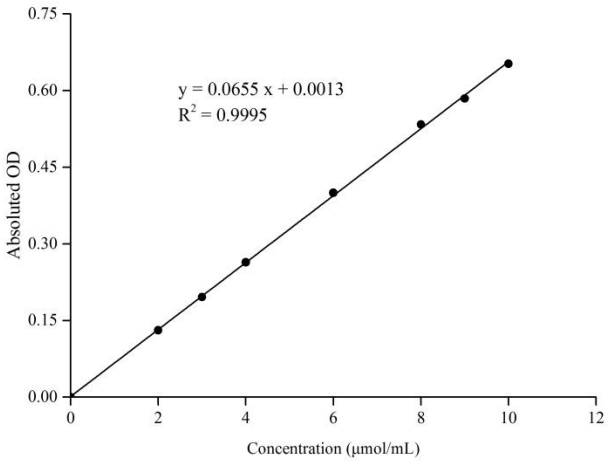
13. Appendix II Example Analysis

Example analysis:

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

Standard curve: y = 0.0655x + 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



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Please follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.