PRODUCT INFORMATION & MANUAL

Ethanol Assay Kit (Colorimetric)

NBP3-25790

For research use only.
Not for diagnostic or therapeutic procedures.

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Ethanol Assay Kit (Colorimetric)

Catalog No: NBP3-25790

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.27 μmol/mL

Detection range: 0.27–10.0 μmol/mL

Average intra-assay CV (%): 3.5

Average inter-assay CV (%): 5

Verage recovery rate (%): 96

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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	Specification	Storage
Reagent 1	Buffer Solution A	20 mL × 1 vial	-20℃ , 12 months
Reagent 2	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months shading light
Reagent 3	Buffer Solution B	14 mL × 1 vial	-20℃ , 12 months
Reagent 4	Substrate	Powder × 2 vials	-20℃ , 12 months shading light
Reagent 5	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 6	10 μmol/mL Standard Solution	1.8 mL × 2 vials	-20℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ 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 reagent 2 with 180 μL 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.

Pre-assay preparation

▲ Reagent preparation

1. Bring reagent 2 on the ice box and the other reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 with 180 μL double distilled water and mix fully. The prepared solution should be stood at room temperature for 30 min before use. The prepared solution can be stored at 2-8℃ with shading light for 4 days.
3. Preparation of reagent 4 working solution:
   Dissolve a vial of reagent 4 with 170 μL double distilled water and mix fully. The prepared solution can be stored at -20℃ with shading light for 4 days.
4. Preparation of reaction working solution:
   Mix the reagent 1, reagent 2 working solution, reagent 3, reagent 4 working solution and reagent 5 at the ratio of 80:1:37:2:8 fully and preserve it with shading light for detection. Prepare the fresh needed amount before use and the prepared solution should be used within 0.5 h.（Preparation of the reaction working solution after the standard and sample are added to the wells）

▲ Sample preparation

Serum and plasma: Detect the sample directly.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.27–10.0 μmol/mL).

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.

Assay protocol

▲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.

▲Detailed operating steps

1. The preparation of standard curve

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.

Number	Standard concentrations (μmol/mL)	17 μmol/mL Standard (μL)	Double distilled water (μL)
A	0	0	200
B	2	40	160
C	3	60	140
D	4	80	120
E	6	120	80
F	8	160	40
G	9	180	20
H	10	200	0

2. 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℃ with shading light for 10 min.

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

▲Summary operation table

	Standard well	Sample well
Standard of different concentrations (μL)	40	
Sample (μL)		40
Reaction working solution (μL)	160	160
Mix fully. Measure the OD value at 450 nm, recorded as A_1.		
Incubate at 37℃ with shading light for 10 min.		
Measure the OD value at 450 nm, recorded as A_2. $\Delta A = A_2 - A_1$.		

▲Calculation

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:

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

Note:

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.

Appendix I Data

▲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