

EZ- Ethanol Assay Kit

Metabolism Assay Kit
(Colorimetric)

Cat. No. DG-ETH100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Ethanol is a colorless, volatile liquid and a type of alcohol that is flammable, soluble, sterilizing, and has a characteristic odor. Ethanol is widely used as alcohol, fuel, solvent, disinfectant, and chemical raw material. Quantitative analysis of ethanol used in various fields can be used for purposes such as quality control, safety assessment, and production optimization.

DoGENBio's EZ-Ethanol Assay Kit is a product that can quickly and accurately measure ethanol concentration in various samples such as serum, plasma, body fluids, food, beverages, and media.

▪ Kit Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage (reconstituted)
Ethanol Assay buffer	25 mL	-	-20 °C, Use within 2 months after opening
Ethanol Enzyme mix (Lyophilized)	1 vial	Red	
Ethanol Probe	200 µL	Yellow	
Ethanol standard (1mM)	1 mL	Green	

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ NOTE

* This product is extremely sensitive to alcohol vapor (ethanol, methanol, propanol), so caution is required. When the kit components are exposed to alcohol vapor, the background increases and the accuracy decreases significantly. Be careful when using alcohol spray for sterilization or cleaning before the experiment. It is recommended to conduct the experiment in a clean bench.

Be sure to read the protocol contents carefully before the experiment and conduct the experiment.

▪ Sample type

- Serum, Biological fluid samples
- Beverage

▪ Preparation of Experiment

- 실험 전 클린벤치를 가동하여 충분히 알코올 증기를 제거합니다.
- 모든 시약은 클린벤치 내에서 개봉합니다.
- 흡광도 측정을 제외하고 모든 실험은 클린벤치 내에서 진행하십시오.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Ethanol Enzyme mix	Add 220 μl Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20°C .
Ethanol Probe	Use after sufficiently dissolving at room temperature.	The remaining solution can be stored at -20°C , but use it within 2 months.
Ethanol Standard	Use after sufficiently dissolving at room temperature.	The remaining solution can be stored at -20°C , but use it within 2 months.

* Assay buffer is used after sufficiently warming up to room temperature before experiment.

* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Standard preparation

After adding 2-50 μl of the prepared sample into a 96 well plate, adjust the final volume to 50 μl with assay buffer. ($n \geq 2$)

Standard No.	Volume of 1 mM Ethanol standard	Assay buffer	Final standard volume in well	Final standard amount in well (nmol/well)
1	0 $\mu\ell$	50 $\mu\ell$	50 $\mu\ell$	0
2	2 $\mu\ell$	48 $\mu\ell$	50 $\mu\ell$	2
3	4 $\mu\ell$	46 $\mu\ell$	50 $\mu\ell$	4
4	6 $\mu\ell$	44 $\mu\ell$	50 $\mu\ell$	6
5	8 $\mu\ell$	42 $\mu\ell$	50 $\mu\ell$	8
6	10 $\mu\ell$	40 $\mu\ell$	50 $\mu\ell$	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2. Sample preparation

1) For unknown samples or samples being measured for the first time, ensure that the measured values are within the standard curve. We recommend using it after preliminary testing.

2) Serum, Biological fluid samples

For high alcohol concentrations (0.01–0.16%, or 1.7–27.4 mM), dilute 1:10–1:100 with assay buffer and use at 20 $\mu\ell$ /well.

3) Beverages

For samples with alcohol concentrations higher than 0.1% (17 mM), dilute 50-fold with assay buffer for every 0.1% and use at 20 $\mu\ell$ /well.

4) Add 20 $\mu\ell$ of the prepared sample to a 96-well plate, and adjust the final volume to 50 $\mu\ell$ with assay buffer. ($n \geq 2$)

3. Reaction mixture preparation

☞ This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

Components	Reaction mixture
Ethanol Assay buffer	46 $\mu\ell$
Ethanol Enzyme mix	2 $\mu\ell$
Ethanol Probe	2 $\mu\ell$
Total	50 $\mu\ell$

4. Add 50 μl of reaction mixture to each well containing the diluted ethanol standard and sample, and mix well. (Using a multi-pipette is recommended.)

5. Incubate the plate in a clean room at room temperature for 60 minutes, blocking light.

After the reaction is complete, gently shake the plate and measure the absorbance at 570 nm.

* After the reaction is complete, the absorbance is measured immediately because exposure to alcohol vapor outside the clean bench may increase the background.

▪ Calculation

1. Subtract the O.D. value of the blank from all measurements.

* Blank = OD_{570nm} from ethanol standard #1 (0 nmol ethanol)

2. Plot the O.D. values (Y-axis) against the Ethanol standard (X-axis) to generate a standard curve and determine the linear equation.

3. Calculate the concentration for each sample using the linear equation represented by the standard curve.

4. Calculate the amount of Ethanol in the sample by substituting the sample measurement value into the standard curve.

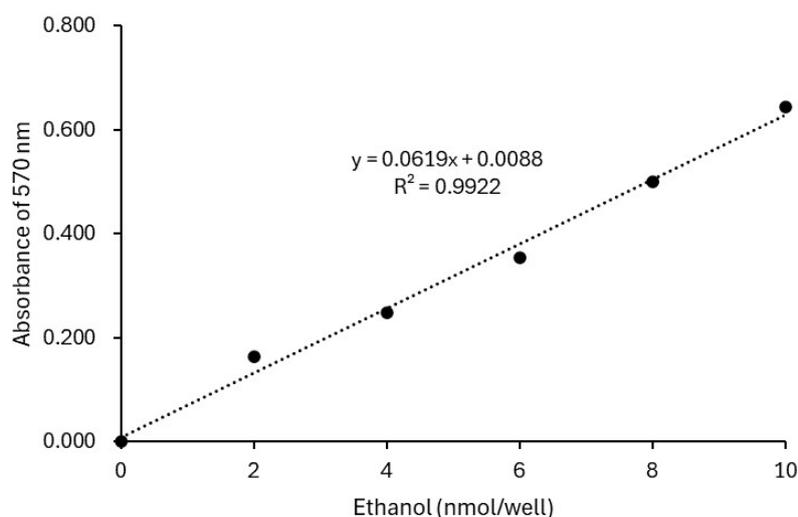
5. Based on the amount of ethanol calculated in 4, calculate the concentration of ethanol in the sample using the following formula.

Ethanol concentration in the sample (C) = B/V x D (nmol/ μl or mM)

B : Amount of ethanol in the measurement well obtained from the standard curve (nmol)

V : Amount of sample in a well (μl)

D : Sample dilution factor (if diluted 2-fold, calculate as x2, not x 1/2)



Ethanol standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
Metabolism Assay Kit	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay

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100 Assay
