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User's Manual and Instructions

Enzyme Ethanol Assay Kit (Z5030040)

Ultrasensitive Colorimetric Determination of Ethanol at 565 nm

DESCRIPTION

Alcoholic drinks are among the daily consumed beverages. Studies have shown heavy alcohol consumption may lead to various forms of liver diseases and to increased mortality rates. Quantitative determination of alcohol (ethanol, C_2H_5OH) has applications in basic research, drug discovery, clinic studies and in the alcoholic industry.

Simple, direct and automation-ready procedures for measuring ethanol concentration are very desirable. BioChain's enzyme ethanol assay kit is based on alcohol dehydrogenase catalyzed oxidation of ethanol, in which the formed NADH is coupled to the formazan (MTT) chromogen. The intensity of the product color, measured at 565 nm, is proportionate to the ethanol concentration in the sample.

APPLICATIONS

Direct Assays: ethanol in serum, plasma, urine and saliva samples.
Pharmacology: effects of drugs on alcohol metabolism.

KEY FEATURES

Sensitive and accurate. Detection limit 0.0008 vol % (140 μM or 8 ppm), linearity up to 0.1% ethanol in 96-well plate assay.
Convenient. The procedure involves adding working reagent, incubating for 30 min and stropping reaction. No 37°C heater is needed.
High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**Enzyme A:**	Lyophilized
NAD Solution:	1 mL	**Enzyme B:**	120 μL
MTT Solution:	1.5 mL	**Enzyme Buffer:**	150 μL
Standard:	1.5 mL 1% Ethanol	**Stop Reagent:**	12ml

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Reconstitute Enzyme A by adding 120 μL Enzyme Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at -20°C and use within 1 month.

Assay Procedure:

1. *Calibration Curve*. Prepare 0.1% alcohol Premix by mixing 25 μL 1% Standard and 225 μL distilled water. Dilute standard as follows. Transfer 10 μL standards into wells of a clear flat-bottom 96-well plate.

No	Premix + H_2O	Vol (μL)	Ethanol (%)
1	100μL + 0μL	100	0.10
2	60μL + 40μL	100	0.06
3	30μL + 70μL	100	0.03
4	0μL + 100μL	100	0

Samples: add 10 μL sample per well in separate wells. *IMPORTANT*: saliva samples should be diluted 10-fold in PBS prior to assay.

2. *Reaction*. For each well of reaction, prepare Working Reagent by mixing 80 μL Assay Buffer, 1 μL Enzyme A, 1 μL Enzyme A, 2.5 μL NAD and 14 μL MTT. Fresh reconstitution is recommended.

Add 90 μL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly. Incubate 30 min at room temperature. Add 100 μL Stop Reagent per well. Tap plate to mix.

3. Read optical density at 565 nm (520-600nm).

4. *Calculation.* Subtract blank (water, #4) from OD values for the standard wells. Plot Standard Curve (ΔOD *vs* Standard ethanol concentrations) to determine the slope. Sample ethanol concentration is calculated,

$$[\text{Ethanol}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \quad (\%)$$

where OD_{SAMPLE} and OD_{BLANK} are the OD_{565nm} values of the sample and blank (water, #4). *n* is the dilution factor (n = 10 for saliva).

Note: if the sample ethanol concentrations are higher than 0.1%, dilute sample in distill water and repeat this assay. Multiply the results by the dilution factor.

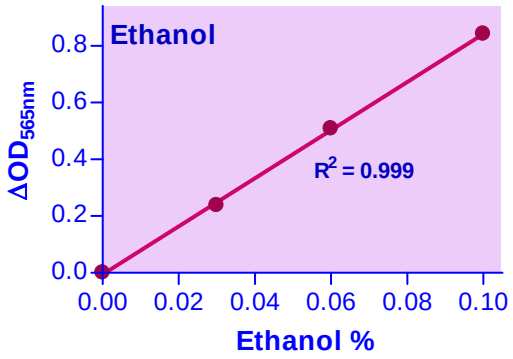
Conversions: 1 vol % ethanol equals 170 mM or 785 mg/dL.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERATIONS

1. This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.
2. The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).



Standard Curve in 96-well plate assay

PUBLICATIONS

1. Dembele, K. et al (2008). Effects of ethanol on pancreatic beta-cell death: interaction with glucose and fatty acids. Cell Biol Toxicol. 25(2):141-52.
2. Ou XM et al (2010). A novel role for glyceraldehyde-3-phosphate dehydrogenase and monoamine oxidase B cascade in ethanol-induced cellular damage. Biol Psychiatry 67(9):855-63.
3. Tapia H and Morano KA (2010). Hsp90 nuclear accumulation in quiescence is linked to chaperone function and spore development in yeast. Mol Biol Cell. 21(1):63-72.