Ethanol Assay Kit

Catalog Number KA4784

100 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

Ultrasensitive Colorimetric Determination of Ethanol at 565 nm

✓ Application

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

✓ 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 stopping 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.

Background

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. 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.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	10 mL
NAD Solution	1 mL
MTT Solution	1.5 mL
Standard (1% ethanol)	1.5 mL
Enzyme A (Lyophilized)	1 vial
Enzyme B	120 µL
Enzyme Buffer	150 µL
Stop Reagent	12 mL

Storage Instruction

Store all reagents at -20°C.

Materials Required but Not Supplied

- ✓ Pipeting (multi-channel) devices
- ✓ Clear flat-bottom 96-well plates (e.g. Corning Costar)
- ✓ Plate reader

Precautions for Use

- ✓ Precautions

  Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

- ✓ General Considerations

- 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.
- 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%).

Assay Protocol

Reagent Preparation

1. Reconstitute Enzyme A by adding 120 µL Enzyme Buffer to the Enzyme A tube.
2. Make sure Enzyme A is fully dissolved by pipetting up and down.
3. 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	100 µL + 0 µL	100	0.10
2	60 µL + 40 µL	100	0.06
3	30 µL + 70 µL	100	0.03
4	0 µL + 100 µL	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 B, 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 (520-600 nm).

Data Analysis

Calculation of Results

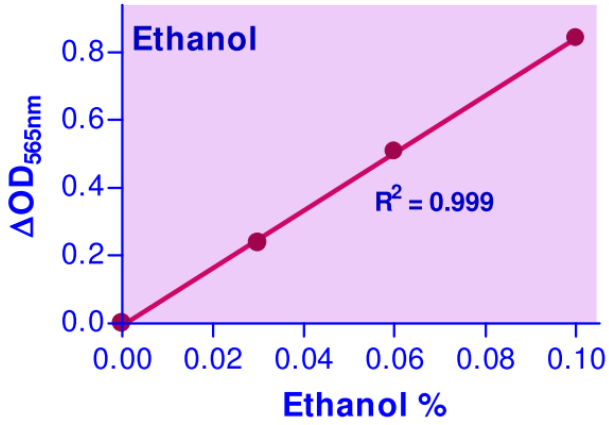
Subtract blank (water, #4) from OD values for the standard wells. Plot Standard Curve (ΔOD vs Standard ethanol concentrations) to determine the slope. Sample ethanol concentration is calculated,

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

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.



Standard Curve in 96-well plate assay