Ethanol Assay Kit (Colorimetric)

LS-K180-500 (500 Tests) • **See Storage Conditions Below**

Introduction

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) finds applications in basic research, drug discovery, clinic studies and winery.

Simple, direct and automation-ready procedures for measuring ethanol concentration are very desirable. This ethanol assay kit is based on an improved dichromate Method, in which dichromate is reduced by ethanol to a bluish chromic (Cr^{3+}) product. The intensity of color, measured at 580 nm, is a direct measure of the alcohol concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits high sensitivity.

Key Features

- Sensitive and accurate. Detection range 0.04 - 4% alcohol in 96-well plate assay.
- Convenient and high-throughput. The procedure involves adding a single working reagent, incubation for 8 min, adding a Stop Reagent, and reading the optical density. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.
- Versatility. Assays can be executed in 96-well plate or cuvette.

Applications

- Ethanol determination in alcohol containing samples such as beverages (e.g. wine, beer) and yeast cultures.

Components

Component	K180-500
	500 Tests
Reagent A	50 mL
Reagent B	50 mL
10% TCA	50 mL
Standard (10% (v/v) ethanol)	2 mL

Materials Not Supplied

Pipetting (multi-channel) devices.

Procedure using 96-well plate: Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette: Centrifuge tubes, table centrifuge, cuvettes and spectrophotometer.

Storage

The kit is shipped at room temperature. Store reagents at room temperature and the ethanol standard at 4°C. Shelf life: 12 months after receipt.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

Procedure using 96-well plate

1. Prepare 600 µL 2% Premix by mixing 120 µL 10% Standard and 480 µL distilled water. Dilute standard as follows. Transfer 100 µL standards and samples into wells of a clear bottom 96-well plate.

No	Premix + H_2O	Vol (µL)	Ethanol (%)
1	150µL + 0µL	150	2.00
2	120µL + 30µL	150	1.60
3	90µL + 60µL	150	1.20
4	60µL + 90µL	150	0.80
5	45µL + 105µL	150	0.60
6	30µL + 120µL	150	0.40
7	15µL + 135µL	150	0.20
8	0µL + 150µL	150	0

2. Add 100 µL Reagent A quickly using a multi-channel pipettor. Tap plate lightly to mix.
3. Incubate 8 to 30 min at room temperature. The reagent color changes from yellow to visibly bluish in wells 1-4. Add 100 µL Stop Reagent B quickly using a multi-channel pipettor. Tap plate to mix.
4. Read OD at 570-600nm (peak 580nm).

Procedure using cuvette

1. Prepare 2%, 1%, 0.5% standards and use distilled water as blank control. Transfer 400 µL diluted Standards and 400 µL samples to 1.5-mL centrifuge tubes.
2. Add 400 µL Reagent A quickly to each tube and vortex briefly to mix.
3. Incubate 8 to 30 min at room temperature. Add 400 µL Reagent B quickly and mix briefly.
4. Transfer to cuvettes and read OD at 570-600nm (peak 580nm).

Note: for the cuvette assay, it is recommended that an interval be applied between additions, e.g., add Reagent A to Tube 1 and 1 min later to Tube 2 etc. After the incubation step is completed, add the Stop Reagent B to Tube 1 and 1 min later to Tube 2 etc. This will ensure identical incubation time between tubes.

Calculations

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard alcohol concentrations. Determine sample ethanol concentration from the standard curve.

Conversions: 1% (v/v) ethanol equals 170 mM or 785 mg/dL.

General Considerations

1. This assay is based on a kinetic reaction. Addition of Reagent A and B (Stop reagent) should be quick and mixing should be brief but thorough.

2. Sample pretreatment. Proteinaceous samples, e.g. plasma, serum, culture media, should be deproteinated by adding 1 vol sample to 2 vol 10% TCA (provided). Pellet for 5 min at 14,000 rpm on a table centrifuge, carefully transfer supernatant for assay (n = 3). Saliva and urine can be analyzed directly (n = 1). For wines, dilute samples to approximately 1 to 2% prior to assay.

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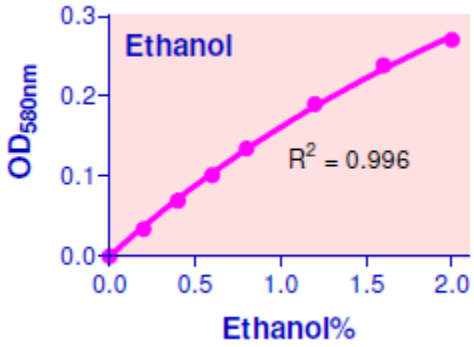
Sample Data



Standard curve in 96-well plate assay

Version: V.08.09.2018

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