QuantiChrom™ Ethanol Assay Kit (DIET-500)

Colorimetric Determination of Ethanol at 580 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) finds applications in basic research, drug discovery, clinic studies and winery.

Simple, direct and automation-ready procedures for measuring ethanol concentration are very desirable. BioAssay Systems' QuantiChrom™ 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.

APPLICATIONS

Ethanol determination in alcohol containing samples such as beverages (e.g. wine, beer) and yeast cultures. For samples containing less than 0.1% alcohol such as serum or plasma, EnzyChrom™ Ethanol Assay Kit (Cat# ECET-100) is recommended.

KEY FEATURES

Sensitive and accurate. Detection range 0.04 – 2% 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 cuvet.

KIT CONTENTS (500 tests in 96-well plates)

Reagent A: 50 mL	**Reagent B: 50 mL**
10% TCA: 50 mL	**Standard: 2 mL 10% (v/v) ethanol**

Storage conditions. 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.

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

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.

CALCULATION

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices.

Procedure using 96-well plate:

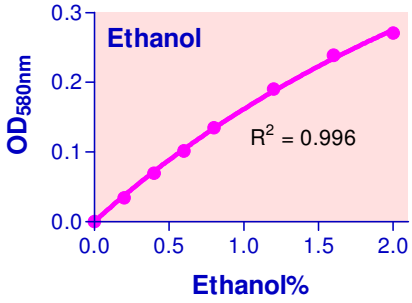
Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Centrifuge tubes, table centrifuge, cuvets and spectrophotometer.

GENERAL CONSIDERATIONS

1. If sample contains glucose or glycerol, use BioAssay Systems' specific ethanol assay kit (ECET-100). For samples containing only sugars (e.g. glucose), use Saccharide Removal Kit (DSRK-500) to remove the interferents prior to assay with the DIET-500 Kit.
2. 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.
3. 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.



STANDARD CURVE IN 96-WELL PLATE ASSAY

PUBLICATIONS

1. Laksitorini, M. D., et al. (2020). Impact of Wnt/beta-catenin signaling on ethanol-induced changes in brain endothelial cell permeability. Journal of Neurochemistry.

2. Duy, D. L., Suda, Y., & Irie, K. (2017). Cytoplasmic deadenylase Ccr4 is required for translational repression of LRG1 mRNA in the stationary phase. PloS one, 12(2).

3. Nugent, B., Ali, S. S., Mullins, E., & Doohan, F. M. (2019). A Major Facilitator Superfamily Peptide Transporter From Fusarium oxysporum Influences Bioethanol Production From Lignocellulosic Material. Frontiers in microbiology 10:295.

Related products EnzyChrom™ Ethanol Assay Kit (ECET-100)
Saccharide Removal Kit (DSRK-500)