Technical Bulletin

Ethanol Assay Kit

Catalogue Number MAK480

Product Description

Alcoholic drinks are among the daily consumed beverages. Studies have shown that heavy alcohol consumption may lead to various forms of liver diseases and to increased mortality rates.

Simple, direct, and automation-ready procedures for the quantitative determination of alcohol (ethanol, C_2H_5OH) finds applications in basic research, drug discovery, clinical studies and the wine industry. The 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.

The linear detection range of the kit is 0.04 to 2.0%. The kit is suitable for the quantitative determination of ethanol in samples such as alcoholic beverages.

Note: For samples containing less than 0.1% alcohol, such as serum or plasma, Ethanol Assay Kit (Catalogue Number MAK481) is recommended.

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Reagent A — 50 mL
  Catalogue Number MAK480A
- Reagent B — 50 mL
  Catalogue Number MAK480B
- 10% TCA — 50 mL
  Catalogue Number MAK480C
- Standard (10% [v/v] Ethanol) — 2 mL
  Catalogue Number MAK480D

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of RCF ≥ 14,000 × *g*

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Reagent A, Reagent B and 10% TCA may be stored at either room temperature or 2-8 °C.

Preparation Instructions

Equilibrate all components to room temperature prior to use. Reagents are ready to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: If sample contains glucose or glycerol, use Ethanol Assay Kit (Catalogue Number MAK481) for ethanol quantification. For samples containing only sugars (such as, glucose), use a saccharide removal kit to remove the interferents prior to assay with this kit.

Sample pretreatment is recommended for proteinaceous samples (for example, plasma, serum, and culture media).

1. Add 1 volume of sample to 2 volumes of 10% TCA (provided).
2. Pellet the protein precipitate by centrifuging for 5 minutes, 14,000 × *g* at room temperature.
3. Carefully transfer supernatant and retain for assay. Dilution factor (DF) = 3.

Saliva and urine can be analyzed directly.

For wines, dilute samples to approximately 1 to 2% prior to assay.

Transfer 100 µL of Sample into separate wells of a clear flat-bottom 96-well plate.

Standard Curve Preparation

Prepare a 2% Ethanol Standard by mixing 120 µL of the 10% Ethanol Standard with 480 µL of purified water. Prepare Ethanol Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Ethanol Standards

Well	2% Ethanol Standard	Purified water	Ethanol (%)
1	150 µL	-	2.0
2	120 µL	30 µL	1.6
3	90 µL	60 µL	1.2
4	60 µL	90 µL	0.8
5	45 µL	105 µL	0.6
6	30 µL	120 µL	0.4
7	15 µL	135 µL	0.2
8	-	150 µL	0

Transfer 100 µL of diluted Standards into wells of a clear 96-well plate.

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Reagent A and Reagent B to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. **Quickly** add 100 µL of Reagent A to Sample and Standard wells. Tap plate lightly to mix.
2. Incubate the plate for 8 to 30 minutes at room temperature. The reagent color changes from yellow to visibly bluish in Standard wells 1-4.
3. At the end of the incubation period, **quickly** add 100 µL of Reagent B to stop the reaction. Tap plate to mix.

Measurement

Read the optical density (OD) at 580 nm.

Results

1. Subtract the Blank (Standard# 8) OD value from the remaining Standard OD values.
2. Plot the corrected OD against standard alcohol concentrations and determine the slope using linear regression fitting.
3. Determine Sample ethanol concentration from the standard curve.

Ethanol (percentage)

$$\frac{OD_{Sample} - OD_{Blank}}{Slope}$$

where:

OD_{Sample} = OD reading of Sample

OD_{Blank} = OD of Blank (Standard #8)

If applicable, multiply the result by the dilution factor (DF).

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

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak480pis Rev 09/22