Technical Bulletin

Ethanol Assay Kit

Catalogue Number MAK481

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 the 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.

The linear detection range of the kit is 0.0008% (v/v) (140 µM or 8 ppm) to 0.1% (v/v). The kit is suitable for the quantitative determination of ethanol in serum, plasma, urine, and saliva samples, as well as studying the effects of drugs on alcohol metabolism.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK481A
- NAD Solution 1 mL
  Catalogue Number MAK481B
- MTT Solution 1.5 mL
  Catalogue Number MAK481C
- Enzyme A 1 vial
  Catalogue Number MAK481D
- Enzyme B 120 µL
  Catalogue Number MAK481E
- Enzyme Buffer 150 µL
  Catalogue Number MAK481F
- Stop Reagent 12 mL
  Catalogue Number MAK481G
- Standard (1% Ethanol) 1.5 mL
  Catalogue Number MAK481H

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

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 on wet ice. Store components at -20 °C.

Preparation Instructions

Enzyme A: Reconstitute vial with 120 µL of Enzyme Buffer. Ensure that Enzyme A is fully dissolved by pipetting up and down.

Store reconstituted Enzyme A at -20 °C and use within 1 month.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: 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%).

Note: Saliva samples should be diluted 10-fold in Phosphate Buffered Saline (PBS) prior to assay.

Transfer 10 µL of sample into separate wells of a clear flat-bottom 96-well plate.

Standard Curve Preparation

1. Prepare a 0.1% Ethanol Standard by mixing 25 µL of the 1% Ethanol Standard with 225 µL of purified water.
2. Prepare Ethanol Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Ethanol Standards

Well	0.1% Ethanol Standard	Purified water	Ethanol (%)
1	100 µL	-	0.10
2	60 µL	40 µL	0.06
3	30 µL	70 µL	0.03
4	-	100 µL	0

3. Transfer 10 µL of diluted Standards into wells of a clear 96-well plate.

Working Reagent

For each well, prepare 98.5 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	80 µL
Enzyme A	1 µL
Enzyme B	1 µL
NAD Solution	2.5 µL
MTT Solution	14 µL

Assay Reaction

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

1. Add 90 µL of Working Reagent to all wells. Tap plate lightly to mix.
2. Incubate the plate for 30 minutes at room temperature.
3. Add 100 µL of Stop Reagent. Tap plate to mix.

Measurement

Read the optical density (OD) at 565 nm.

Results

1. Subtract the Blank (Standard# 4) OD value from the remaining standard OD values (ΔOD).
2. Plot the standard curve (ΔOD versus Standard ethanol concentrations) and determine the slope using linear regression fitting.
3. Determine sample ethanol concentration from the standard curve.

   % Ethanol =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope} \; x \; DF$$

where:

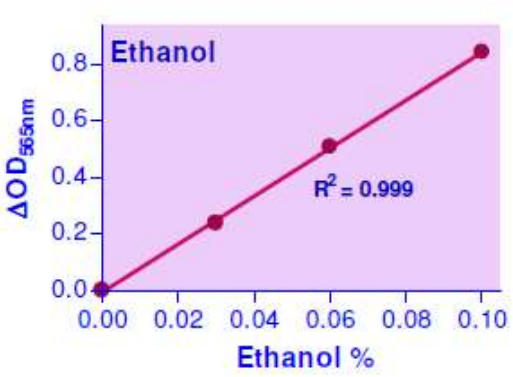
OD_{Sample} = OD reading of Sample at 565 nm

OD_{Blank} = OD reading of Blank (Standard #4) at 565 nm

DF = Sample dilution factor (DF = 1 for undiluted Samples. Sample dilution factor for saliva is 10.)

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

Typical Ethanol Standard Curve



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.

mak481pis Rev 09/22