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Product Information

Ethanol Assay Kit

Catalog Number **MAK076**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Ethanol is a psychoactive component of many commonly consumed drinks where it acts as a central nervous system depressant. After ingestion, ethanol is absorbed into the bloodstream via the stomach and small intestine. Ethanol is largely metabolized by the liver but is also secreted in urine or through respiration. The monitoring of ethanol levels is also important in fermentation processes.

The Ethanol Assay Kit provides a simple and reliable method for the quantification of ethanol in serum, plasma, and other body fluids as well as in beverages and growth media. Ethanol concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the ethanol present.

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

Ethanol Assay Buffer Catalog Number MAK076A	25 mL
Ethanol Probe, in DMSO Catalog Number MAK076B	0.2 mL
Ethanol Enzyme Mix Catalog Number MAK076C	1 vl
Ethanol Standard, 17.15 N Catalog Number MAK076D	0.5 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is 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.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Ethanol Assay Buffer – Allow buffer to come to room temperature before use.

Ethanol Probe – Warm to room temperature to melt frozen solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the Ethanol Probe is ready to use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Ethanol Probe Solution 5 to 10-fold with Ethanol Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Ethanol Enzyme Mix – Reconstitute in 220 µL of Ethanol Assay Buffer. Mix well by pipetting, then store, protected from light, at 2–8 °C. Stable for 2 months.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Note: This kit is highly sensitive to the presence of short-chain alcohols (ethanol, methanol, and propanol). Storage of this kit in the vicinity of alcohol vapors can result in the uptake of the alcohols by kit components, resulting in very high backgrounds.

Procedure
This assay should not be performed in areas where alcohol-containing solvents are stored or where laboratory benches are wiped down with alcohol.

All samples and standards should be run in duplicate.

Ethanol Standards for Colorimetric Detection
Dilute 50 µL of the 17.15 N Ethanol Standard with 808.7 µL of the Ethanol Assay Buffer to generate a 1 µmole/µL standard. Dilute 10 µL of the 1 µmole/µL standard solution with 990 µL of ethanol assay buffer to generate a 10 nmole/µL solution. Dilute 100 µL of the 10 nmole/µL solution with 900 µL to generate a 1 nmole/µL ethanol standard. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Ethanol Assay Buffer to each well to bring the volume to 50 µL.

Ethanol Standards for Fluorometric Detection
Generate a 10 nmole/µL ethanol standard solution as for the colorimetric assay. Dilute 10 µL of the 10 nmole/µL ethanol standard with 990 µL of the Ethanol Assay Buffer to generate a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Ethanol Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Samples should be diluted in Ethanol Assay Buffer.

Bring samples to a final volume of 50 µL with Ethanol Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mix

Reagent	**Volume**
Ethanol Assay Buffer	46 µL
Ethanol Probe	2 µL
Ethanol Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C or 60 minutes at room temperature. Cover the plate tightly and protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Ethanol Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate ethanol standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of ethanol present in the sample may be determined from the standard curve.

Concentration of Ethanol

$$S_a/S_v = C$$

S_a = Amount of ethanol in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of ethanol in sample

Ethanol molecular weight: 46.07 g/mole

Sample Calculation

Amount of ethanol (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of ethanol in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 46.07 \text{ ng/nmole} = 5.38 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Ethanol Assay Buffer
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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