ETHANOL
(LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007710
K-ETOHLQ

08/25

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

Megazyme® by NEOGEN®

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FS02312_0825

INTRODUCTION:

Ethanol is ubiquitous in its natural occurrence, and thus its quantitative determination is not only important in the manufacture of wines, beers, and spirits, but also for low-alcohol and non-alcoholic beverages, fruit juices and a range of other foodstuffs, including chocolates, sweets, jam, honey, vinegar, and dairy products.

PRINCIPLE:

Alcohol dehydrogenase (AlDH) catalyzes the oxidation of ethanol to acetaldehyde coupled with the reduction of nicotinamide-adenine dinucleotide (NAD^+).

$$Ethanol + NAD^+ \xrightarrow{AlDH} Acetaldehyde + NADH + H^+$$

The amount of NADH formed in this reaction pathway is stoichiometric with the amount of Ethanol. It is the NADH which is measured by the increase in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific to Ethanol.
- The limit of detection (LOD) is 0.002 g/L, and the limit of quantification (LOQ) is 0.005 g/L (using a sample volume of 0.1 mL).
- The recommended measuring range is between 0.01 and 0.3 g/L (using a sample volume of 0.1 mL). This corresponds to 1 - 30 µg of Ethanol per assay.

INTERFERENCE:

No interfering compounds have been identified.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents may be disposed of with standard laboratory waste, in accordance with local regulations and guidelines.

NOTE: For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KIT CONTENTS:

Kits are designed for use in both manual and automated workflows. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL): Buffer
Contains sodium azide (0.02% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Reagent 2 (2 x 12.5 mL): NAD^+, AlDH
Contains sodium azide (0.02% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Standard (5 mL): Ethanol standard (0.3 g/L).
Contains sodium azide (0.02% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

NOTE: The Ethanol standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of Ethanol is determined directly from the extinction coefficient of NADH.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25 °C) before use.

MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 2.60 mL
Sample solution: 0.01 g/L to 0.3 g/L (i.e. 1 - 30 µg of Ethanol per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 minutes at 20 - 37°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~ 7 minutes at 20 -37°C, then read the absorbances (A_2).**		

* Either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.
** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 minute intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

NOTE: The reagent blank value must be determined once for each run and subtracted from each sample result.

CALCULATION:

NOTE: These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) based on the component ratios:

$$df = \frac{\text{Sample volume [mL] + R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the Ethanol manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{Ethanol}$

$$\Delta A_{Ethanol} = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{blank}$$

It follows for the Ethanol manual assay procedure:

$$\Delta A_{Ethanol} = (A_2 - 0.808 \times A_1)_{sample} - (A_2 - 0.808 \times A_1)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor; if volumes are changed, the system and performance may be affected.

3. Calculation of the Ethanol content in g/L

The concentration of Ethanol can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{Ethanol} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of Ethanol [g/mol]
ε = extinction coefficient of NADH at 340 nm [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for the Ethanol manual assay procedure:

$$c = \frac{2.6 \times 46.07}{6300 \times 1.0 \times 0.1} \times \Delta A_{Ethanol} \quad [g/L]$$

$$= 0.1901 \times \Delta A_{Ethanol} \quad [g/L]$$

4. Calculation of the Ethanol content in % (v/v) terms:

To calculate alcohol-by-volume % (v/v) for ethanol:

$$c = \frac{2.6 \times 46.07}{6300 \times 1.0 \times 0.1} \times 0.1266 \times \Delta A_{Ethanol} \quad [\% \textit{ v/v}]$$

$$= 0.0241 \times \Delta A_{Ethanol} \quad [\% \textit{ v/v}]$$

where:
0.1266 = factor to convert g/L to % (*v/v*), taking the density of pure ethanol to be 0.79 g/mL

If the sample has been diluted during preparation, the result must be multiplied by the sample dilution factor, F.

5. Calculation of the Ethanol content in solid or semi-solid samples:

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

$$\frac{C_{Ethanol} \text{ [g/L sample solution]}}{\text{weight}_{sample} \text{ [g/L sample solution]}} \times 100 \quad [g/100g]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for auto-analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

NOTE: For each batch of samples that is applied to the determination of Ethanol a calibration curve must be created concurrently using the same batch of reagents.

Parameter	Details
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1-3 minutes [20 - 37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Incubate 7 minutes at [20 - 37°C] – Measure A_2 at 340/405 nm – Calculate $A_2 - A_1$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 0.3 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.03 g/L (dilute Standard 10-fold) Calibrator 3 0.15 g/L (dilute Standard 2-fold) Calibrator 4 0.3 g/L (use Standard as-is) *Perform all dilutions with distilled water.*

SAMPLE PREPARATION:

1. Sample dilution

The amount of Ethanol present in the sample should range from 0.01 g/L to 0.3 g/L. If the value of $\Delta A_{Ethanol}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value $\Delta A_{Ethanol}$ is too high (e.g. >2.0), increase the dilution in distilled water.

Sample Dilution Table

Estimated Concentration of Ethanol (g/L)	Dilution with Water	Sample Dilution factor (F)
< 0.3	No dilution required	1
0.3 – 3	1 mL sample + 9 mL water	10
3 -30	1 mL sample + 99 mL water	100

2. General sample preparation guide

- Clear, slightly colored, and approximately neutral, liquid samples at a concentration up to 0.3 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH < 3.0) must be neutralized to approximately pH 8.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenized, extracted in water, and filtered or centrifuged if necessary.
- Strongly colored samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinize samples using the Carrez Clarification Kit (700004270, K-CARREZ).
- Remove fat using the Carrez Clarification Kit (700004270, K-CARREZ).

3. Suggested sample preparation examples

(a) Determination of Ethanol in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, a 1000-fold dilution in distilled water is required for red wine and no dilution is required for white wine.*

(b) Determination of Ethanol in "alcohol-free" beers: Remove carbonation by stirring a sample in a beaker and increasing the pH to approximately pH 9 using 2 M sodium hydroxide. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, 20-fold dilution is required.*

(c) Determination of Ethanol in "alcohol-free" spirits (e.g. alcohol-free gin): The Ethanol concentration of alcohol-free spirits can generally be determined without any sample treatment (except dilution according to the dilution table). *Typically, a 10-fold dilution in distilled water is required*

(d) Determination of Ethanol in raw unpasteurized kombucha. Remove carbonation by stirring a sample in a beaker and increasing the pH to approximately pH 9 using 2 M sodium hydroxide. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, 10-fold dilution is required.*

(e) Determination of Ethanol in fruit juice (e.g. tomato juice): Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, 2-fold dilution is required.*

IMPORTANT NOTE: The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

Troubleshooting
Data analysis
Additional matrix testing
Application support in relation to automated analyzers

Supporting documents can be found on the product page:

Quick Reference Guide
MegaCalc™
Safety Data Sheets (SDS)
Certificates Of Analysis (COA)
Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

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User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique, and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

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