

Enzymatic determination of ethanol using alcohol oxidase and peroxidase with LCK300

Background

Why Measure Alcohol?

Accurate determination of ethanol content is essential in multiple sectors:

· Regulatory Compliance:

Alcohol content in beverages is subject to strict legal limits. For example, "alcohol-free" beers in many regions must contain <0.5 vol% ethanol. Reliable testing ensures compliance with labeling laws.

· Quality Control in Production:

In breweries, wineries, and juice production, ethanol levels are monitored to maintain consistent product quality and flavor. Undesired fermentation during storage can lead to unexpected alcohol formation, especially in juices.

· Consumer Safety:

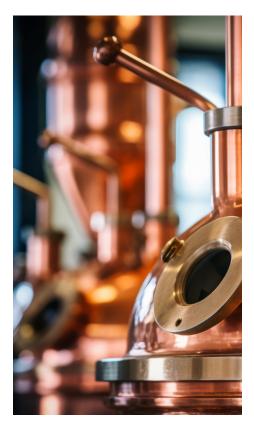
Some consumers avoid alcohol for health, religious, or personal reasons. Accurate measurement ensures transparency and trust in product claims.

· Process Optimization:

In fermentation-based industries, ethanol measurement helps monitor reaction progress, determine optimal harvest points, and minimize waste.

· Research and Development:

Food scientists and beverage technologists rely on ethanol testing to develop new products, such as low-alcohol beers or alcohol-free cocktails, ensuring desired sensory profiles without exceeding target ethanol levels.



Scope and Application

- **Intended Samples:** Spirits, beers, alcohol-free beers, fruit juices
- · Typical Applications:
 - Fruit juices
 Detect very low ethanol levels
 (0.05–0.50 g/L)
 - Alcohol-free beer (<0.5 vol%)
 Ensure compliance with labeling claims
 - Low-alcohol beer Measure mid-range ethanol concentrations
 - Strong alcoholic beverages

 Quantify high ethanol contents up to 60 vol% (after dilution)

Sample Requirements

Field of Application	Fruit Juices	Alcohol- free Beer	Low-alcohol Beer	Strong- alcohol
		< .05% vol	"Lite Beer," Medium gravity beer	Strong beer, wine, spirits
Estimated Alcohol Content (g/L)	0.05-0.50	0.5-5.0	5–50	50-500
Estimated Alcohol Content (Vol. %)	0.006-0.06	0.06-0.6	0.6-6.0	6-60
Dilution Factor	1:5	1:50	1:500	1:5000
Preliminary Dilution*	none	none	5mL sample	0.5mL sample
Dilution*	10 mL sample	1mL sample	1ml preliminary dilution	1ml preliminary dilution

^{*} Sample volume/preliminary dilution in a 50 mL measuring flask

[→] Conversion of the reading into vol %: displayed result (g/L) x dilution factor x 0.126

Interference Control

- Avoid oxidizing agents They cause false results.
- **Reducing agents (e.g., ascorbic acid)** Tolerated up to 20 mg/L.
- **Filtration** Required for turbid or particulate-rich samples (membrane LCW904).
- **Degassing** For CO₂-containing samples, stir 1 minute before analysis.
- **pH Adjustment** Necessary for highly acidic fruit juices.

Principle of the Method

Ethanol oxidation:

The enzyme alcohol oxidase catalyses the following reaction:

Ethanol + H₂O + O₂ Alcohol oxidase Acetaldehyde + H₂O₂

The resulting hydrogen-peroxide combines with aminophenazone and benzoic acid derivative in the presence of peroxidase, which acts as a catalyst, to form a red quinoid dye.

 H_2O_2 + Aminophenazone + Benzoic acid derivative $\xrightarrow{Peroxidase}$ Red quinoid dye

Photometric measurement

Absorbance is directly proportional to ethanol concentration.

Test Procedure

Product Code: LCK300

Measurement Range: 0.01–0.12 g/L alcohol (C₂H₅OH) before dilution

- 1. Attach a **DosiCap A** to the cuvette, invert to mix.
- 2. Pipette **0.2 mL of diluted sample** into the same cuvette.
- 3. Close and invert several times.
- 4. After 30 minutes, invert again, clean the cuvette exterior.
- 5 **Zero** the instrument with the blank cuvette, insert sample cuvette, and read result.

Performance and Quality Control

- Ensure storage of kits at 2-8 °C.
- Check expiry date before use.
- Validate results by **testing multiple dilutions** or spiking samples.
- Dispose of reagents per local regulations and SDS guidelines.

Key Advantages

- Applicable across wide ethanol concentration range via dilution.
- **High specificity** for ethanol due to enzyme-based reaction.
- Suitable for quality control in beverages and juices.



Alcohol cuvette test 0.01-0.12 g/L, 24 tests



DR1900 Portable Spectrophotometer



DR3900 Laboratory VIS Spectrophotometer with RFID* Technology



DR6000 UV VIS Spectrophotometer with RFID Technology

