

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:



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.



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