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.



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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 CO_2-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:

$$\text{Ethanol} + H_2O + O_2 \xrightarrow{\text{Alcohol oxidase}} \text{Acetaldehyde} + H_2O_2$$

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.

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

Photometric measurement

Absorbance is directly proportional to ethanol concentration.

Test Procedure

Product Code: LCK300
Measurement Range: 0.01–0.12 g/L alcohol (C_2H_5OH) 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

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