Determination of Ethanol in food products
Test-kit for 32 determinations on the RIDA®CUBE SCAN instrument (340 nm)

For in vitro use only
Store between +2 and +8°C

Principle

Enzymatic test with Alcohol Dehydrogenase (ADH). NADH is produced and is measured at 340 nm:

Ethanol + NAD^+ —— ADH ⟶ Acetaldehyd + NADH + H^+

Reagents

1: 32 tubes with approx. 800 µl reagent 1 (buffer)

2: 32 caps with approx. 200 µl reagent 2 (enzyme)

3: One RFID card (Radio Frequency Identification)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS), available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

Because Ethanol is volatile, it is necessary to follow specific rules, otherwise the recovery will be low:

- When diluting sample solution, pipeting of the sample must be always under the surface of the dilution solution
- When filtering sample solution, the filtrate has not to drop, but rinse down the wall of the container
- Perform all steps in a closed vial, open when necessary

General sample preparation procedures:

- Use clear, liquid samples directly, or after dilution into the relevant measuring range
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Clarify samples containing proteins or fat with Carrez clarification
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge, or use Carrez clarification if necessary
- For fat containing samples, weigh sample into a volumetric flask (min. 50 ml) and extract with hot water; cool to allow the fat to separate (for example on an ice bath for 15 min). Make up to the mark with water, remove the fatty layer on the top and filter the aqueous part before testing.

Assay specifications

The test instructions are saved on the RFID card and are executed automatically by the instrument.

Wavelength:	340 nm
Temperature:	37 °C
Calibration:	Saved on the RFID card
Test sequence:	Sample + R1 / mix / 2 min / A1 / R2 / mix / 10 min / A2
Sample volume:	20 µl (basic) or 100 µl (sensitive) The selected volume must be pipeted precisely into reagent 1 (test tube).

Handling procedure

Place the RFID Card on the instrument	
Enter sample data into tablet app: - identification - vol. (20 or 100 µl)	
Pipette the sample into the test-tube (reagent 1)	 
Close the tube with the cap (reagent 2), insert it into the instrument and close the door	 

Results

The results are given in mg/l by the instrument, and following ranges are recommended:
- from 20 to 500 mg/L for the basic application (20 µl)
- from 4 to 110 mg/L for the sensitive application (100 µl)

The sample volume is 20 µl or 100 µl. For the sensitive application (100 µl), interferences from the sample matrix may occur because of the high sample volume. In this case the samples must be pre-diluted, or they must be diluted directly into the cuvette (e.g. 50 µl sample and 50 µl water). The total volume must stay at 100 µl, and results must be recalculated according to the dilution factor.

Notes

1. Use a quality control every day where a run is performed (e.g. Enzytec Alcohol Standard E5420). If the deviation of this quality control is higher than 10%, it is necessary to measure the reagent blank with a water sample, and to subtract it from all future sample results.
2. The assay is very sensitive. Ethanol from the air could cause false positive results, so it is necessary to run the assay in a room which is free from Alcohol.