Enzymatic assay for Ethanol in foodstuff and other sample materials
x 50 ml R1 + 2 x 12.5 ml R2 (50 assays)

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⟶ Acetaldehyde + NADH + H^+

Reagents

The reagents are ready-to-use.
Reagent 1: two vials ≥ 50 ml (buffer)
Reagent 2: two vials ≥ 12.5 ml (NAD, ADH)

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 further hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, 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

- Use clear, colourless and pH-neutral liquid samples directly, or after dilution into the relevant measuring range (see test performance)
- 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; make up to the mark with water, remove the fatty layer on the top and filter the aqueous part

Assay procedure

Wavelength:	340 nm
Optical path:	1 cm
Temperature:	20 – 25 °C / 37 °C
Measurement:	Against air or against water
Sample solution:	3 – 500 mg/l

	Reagent Blank (RB)	**Samples**
Reagent 1	2000 µl	2000 µl
Sample / Standard	-	100 µl
Dist. water	100 µl	-
Mix, incubate for 1 min. at 37 °C or 3 min. at 20 - 25 °C. Read absorbance A1 , then add:		
Reagent 2	500 µl	500 µl
Mix, wait until the end of the reaction (incubation for approx. 10 min. at 37°C or approx. 15 min. at 20 - 25 °C). Read absorbance A2.		

Reagent blank must be performed once for every run, and subtracted from every sample during calculation of results.

In order to avoid evaporation, the sample must be pipeted into the reagent 1 dispensed before.

Calculation of results

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
With df = dilution factor of optical densities through reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.808

c = (V x MW x Δ A) / (ε x d x v x 1000) [g/l of Ethanol] **c** = (2.600 x 46.07 x Δ A) / (ε x 1 x 0.1 x 1000)
It results for the determination at 340 nm (ε = 6.3 $l.mmol^{-1}.cm^{-1}$):

$$C_{Ethanol}\,[g/l] = \mathbf{0.190} \times \Delta A$$

Calculation in solid samples:

$$Content_{Analyte}\,[g/100\,g] = \frac{C_{Analyte}\,[g/l]}{weight_{sample}\,[g/l]} \times 100$$

Notes

The assay is very sensitive. Ethanol from air (e.g. disinfection or cleaning agents) causes a creep reactions and false results. It is necessary to run the assay in Ethanol free air, or to work with closed (air dense) cuvettes.

Because of the very volatile characteristics of Ethanol it is necessary to follow special procedures, otherwise the recovery will be affected:

- 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.

Test performance

Specificity

ADH oxidizes primary alcohols. The recovery of Ethanol is around 100%, whereas other primary alcohols (n-Propanol and n-butanol) show a lower recovery. Secondary and tertiary alcohols can lead to a creep reaction.

Linearity and measuring range

The test is linear up to 500 mg/l Ethanol. The recommended measuring range lies between 20 and 300 mg/l, in order to keep ΔA ≅ 1.5 (A). When values exceed this range, samples should be diluted into the range 50 – 300 mg/l with dist. water. The dilution factor has to be considered in the calculation.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

- LoD = 1.7 mg/l -
  LoQ = 3.0 mg/l

Automation

Application sheets for automated systems are available on request.

Disclaimer

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