Enzymatic assay for the determination of ethanol in foodstuff and other sample materials
2 x 50 mL R1 and 2 x 12.5 mL R2 – 50 assays (manual) / ≥ 500 assays (auto-analyzer)

For *in vitro* use only
Store between 2 - 8 °C

## Method

Enzymatic UV determination of with alcohol-dehydrogenase (A-DH). AOAC® *Official Method℠* 2017.07 for kombucha, juices, and alcohol-free beer.

## Principle

In the presence of the enzyme alcohol dehydrogenase (A-DH), ethanol is oxidized to acetaldehyde by nicotinamide adenine dinucleotide (NAD). NADH is produced and measured at 340 nm:

Ethanol + $NAD^+$ ——A-DH⟶ Acetaldehyde + NADH + $H^+$

## Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (buffer)
- Reagent 2: 2 x 12.5 mL (NAD, A-DH)

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) 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 liquid and clear samples directly, or after dilution into the relevant measuring range (see test performance).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Clarify protein-containing samples with Carrez reagents.
- Crush and homogenize solid or semi-solid samples and extract with water (e.g. 30 min at 60 °C). Filter or centrifuge, apply Carrez clarification if necessary.
- Extract samples with a high fat content with hot water, then allow to cool (ice or refrigerator) for fat separation. Remove fat layer and filter aqueous solution.
- When diluting the sample solution, always pipette the sample below the surface of the dilution solution.
- During filtration, do not let the filtrate drip, but let it run off the wall of the collection vessel.
- Use diluted samples within the same day.
- Detailed sample preparation guideline available on request.

## Assay procedure

Wavelength: 340 nm
Temperature: 37 °C or 20 - 25 °C
Measurement: against air or against water
Sample: 30 - 300 mg/L

| | Reagent blank | Samples / Controls |
|---|---|---|
| **Reagent 1** | 2000 µL | 2000 µL |
| **Sample / Control** | - | 100 µL |
| **Dist. water** | 100 µL | - |
| Always submit R1! Mix, incubate for 3 min at 37 °C or at 20 - 25 °C. Read absorbance $A_1$, then add: | | |
| **Reagent 2** | 500 µL | 500 µL |
| Mix, wait for the end of the reaction (incubate approx. 10 min at 37 °C or approx. 15 min. at 20 - 25 °C). Read absorbance $A_2$. | | |

The reagent blank value must be determined once for each run and subtracted from each sample result.

## Calculation of results

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: (reagent) dilution factor
RB: Reagent blank

$$df_{basic\ application} = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor.

$$c_{Ethanol}\,[g/L] = \frac{(V \times MG \times \Delta E)}{(\varepsilon \times d \times v \times 1000)}$$

| | | |
|---|---|---|
| V: | Test volume basic application [mL] | = 2.600 |
| MW: | Molecular weight [g/mol] | = 46.07 |
| d: | Optical path [cm] | = 1.00 |
| v: | Sample volume [mL] | = 0.100 |
| ε: | Extinction coefficient NADH [L/mmol x cm] | = 6.3 (at 340 nm) |

For a determination at 340 nm this results in:

**$c_{Ethanol}$ [g/L] = 0.190 x ΔA**

***Alcohol by volume:***

ABV % = $c_{Ethanol}$ [g/L] / 7.8924 (at 20 °C)

## Notes

- Due to the high sensitivity, it is essential to work in an ethanol-free environment or with airtight cuvettes.
- The sample volume should be at least 100 µL.
- Precision is strongly dependent on the pipetting technique.

## Test performance

***Specificity***

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

***Linearity and measuring range***

Linearity is given up to 500 mg/L ethanol. If the recommended measuring range of 30 to 300 mg/L is exceeded, the samples should be diluted with dist. water to a concentration within the measuring range. The dilution factor has to be considered in the calculation.

***Sensitivity***

The limit of detection (LoD) and the limit of quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution for a sample volume of v = 100 µL.
This results in an LoD of 1.9 mg/L and an LoQ of 3.3 mg/L.

***Automation & Validation reports***

Application sheets for automated systems and customer validation reports are available on request.

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