UV assay for the determination of ethanol in kombucha, juices, and alcohol-free beer
AOAC Official Method℠ 2017.07
Test combination for 50 determinations

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

1. Test principle

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

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^+

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

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

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

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 safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbon dioxide by a short burst of ultrasound at 0 °C (ultrasonic device filled with ice cubes and distilled water).
- Ethanol is very volatile; therefore, when diluting sample solutions, always pipette beneath the surface of the diluent; when filtering a sample solution, the filtrate shall not drop but rinse down the wall of the vial; close vial tightly before centrifugation.
- Samples with 0.076 - 0.76 % ABV (0.6 up to 6 g/L ethanol) should be diluted 1 + 19 with water, e.g. 100 µL sample is pipetted into 1900 µL distilled water.
- Samples with 0.38 - 3.8 % ABV (3 up to 30 g/L ethanol) should be diluted 1 + 99 with water, e.g. 100 µL sample is pipetted into 9.90 mL distilled water.
- Other dilutions as, e.g. 1:50 or 1:10, are possible if the ethanol concentration of the diluted samples lies within the measurement range (0.03 up to 0.3 g/L).
- Dilution of ethanol-containing samples with water is very susceptible to pipetted volumes used for dilution. Therefore, pipette at minimum 100 µL ethanol-containing sample into the respected volume of water; lower volumes, e.g. 20 µL, will result in higher CVs.
- Use diluted sample solutions within 3 days for ethanol measurement (storage temperature 4 °C).

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 30 - 300 mg/L

	Reagent blank	**Sample / control**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 15 min at 20 - 37 °C and read absorbance A_2.		

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

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of Ethanol

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$\mathbf{C_{ethanol}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.190 \times \Delta A}$$

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)

Alcohol by volume:

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

Notes:

- Always submit R1!
- 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.

5.2. Calculation of solid samples

$$\text{Content}_{\text{ethanol}}\ [\text{g}/100\ \text{g}] = \frac{C_{\text{ethanol}}\ [\text{g/L sample solution}]}{\text{weight}_{\text{sample}}\ \text{in g/L sample solution}} \times 100$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of alcohol standard (AQ03-015, 300 mg/L ethanol).

The recovery of alcohol standard and other control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity & side activities

Alcohol-dehydrogenase 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.

6.2. Interferences

Acetaldehyde does not interfere at concentrations below 3000 mg/L. Sulfite does not interfere at concentrations below 300 mg/L.

6.3. Linearity, measuring range & sensitivity

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.

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.

6.4. Further applications

The method has been validated for kombucha, juices and non-alcoholic beer. Other foodstuffs and sample materials can be analyzed with this method but require additional validation.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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