AK0006_IFU_EN_V2303

# Ethanol, UV method

| **Catalogue number** | **Presentation** |
| --- | --- |
| AK00061 | 60 tests (manual) / 600 tests (microplate) |

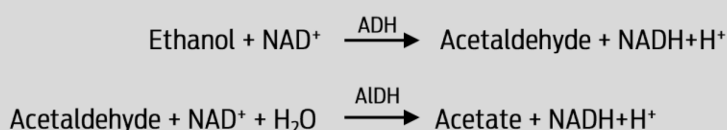
## Application

This rapid and simple specific enzymatic method is used for the determination of ethanol in foodstuffs such as alcoholic and non-alcoholic beverages, bread, dairy products, fruit and vegetables, as well as in cosmetics, pharmaceuticals and biological samples.

## Introduction

Ethanol is ubiquitous in nature. It is the end product of alcoholic fermentation and a key component of alcoholic beverages (although it constitutes an "undesirable" component of non-alcoholic and low-alcoholic beverages). The presence of ethanol in fruit products like fruit juices indicates that the components used for production may have decomposed. The presence of ethanol is also an indirect indicator for the presence of yeasts. A large range of non-foods also contain significant quantities of ethanol, such as cosmetics and pharmaceuticals.

## Principle




The amount of NADH formed through the combined action of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AlDH), measured at 340 nm, is stoichiometric with twice the amount of ethanol in sample volume.

## Specificity

The determination is relatively specific for ethanol, because the content of n-propanol and n-butanol, which also react in the determination, in foodstuffs is so small that it can be neglected. Methanol, aldehydes, ketones, secondary and tertiary alcohols, as well as glycerol do not interfere with the determination.

## Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 AU and a sample volume of 2.00 mL. This corresponds to an ethanol concentration of 0.023 mg/L sample solution when measured at 340 nm. The detection limit of 0.093 mg/L is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume of 2.00 mL.

## Linearity and precision

Linearity of the determination exists from 0.25 to 12 µg ethanol per assay (v = 2.00 mL). In a double assay using one sample solution, a difference of 0.005 to 0.010 AU may occur (0.023-0.046 mg/L of ethanol, v = 2.00 mL). The CV is approx. 1 to 3% in the measuring range.

## Kit composition

**Solution 1.** Potassium pyrophosphate buffer (15 mL, 1.5 M, pH 9.0) and sodium azide (0.02% w/v) as a preservative. Store at 2 °C to 8 °C.

**Solution 2.** $NAD^+$ (155 mg). Store at 2 °C to 8°C (Long term storage: -30 °C to -15 °C)

Dissolve in 12.4 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -30 °C to -15 °C between use and keep cool during use.

**Suspension 3.** Aldehyde dehydrogenase (AlDH, EC 1.2.1.3) in 3.2 M ammonium sulphate (1.3 mL). Store at 2 °C to 8 °C. Swirl bottle before use.

**Suspension 4.** Alcohol dehydrogenase (ADH, EC 1.1.1.1) in 3.2 M ammonium sulphate (1.3 mL). Store at 2 °C to 8 °C. Swirl bottle before use.

**Solution 5.** Ethanol standard solution (5 mL, 5.0 mg/mL). Store at 2 °C to 8 °C.

Dilute 0.5 mL to 50 mL with distilled water; store in a well-sealed bottle and use within 2 days at 2 °C to 8 °C. This standard can be used when there is some doubt about the method accuracy.

## Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic with cap)

Temperature: ~20-25 °C

Final volume: 2.54 mL

Sample solution: 0.25-12 µg of ethanol per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

| PIPETTE INTO CUVETTES (mL) | **BLANK** | **SAMPLE** |
|---|---|---|
| Distilled water (at ~25 °C) | 2.10 | 2.00 |
| Sample | - | 0.10 |
| Solution 1 (PPi buffer) | 0.20 | 0.20 |
| Solution 2 ($NAD^+$) | 0.20 | 0.20 |
| Suspension 3 (Al-DH) | 0.02 | 0.02 |
| Mix, measure the absorbance of the solutions (A1) after ~2 min and start the reaction by addition of | | |
| Suspension 4 (ADH) | 0.02 | 0.02 |
| Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 5 min)* | | |

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* if necessary, continue to read the absorbances at 2 min intervals until the reaction ends.

## Calculation

Determine the absorbance difference for both blank and sample (A2-A1). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{Ethanol}$. The concentration of ethanol, based on the ε of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

$$C\ (Ethanol) = 0.09287 \times \Delta A_{Ethanol} \quad [g/L]$$

$$C\ (Ethanol) = 0.01176 \times \Delta A_{Ethanol} \quad [\%v/v]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

## Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the "Alternative Procedures", available on the NZYtech website.

## Interferences

Commonly, laboratory distilled water, buffers and air are contaminated with ethanol, which can result in increased blanks or in creep reactions. Thus, it is necessary to cover the cuvettes during assay. Eventually, for uncoloured samples, reaction may be started by addition of sample. If the conversion of ethanol has been completed within the time specified (approx. 5 min), it can be generally concluded that no interference has occurred. Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments i.e. by adding ethanol to the sample in the initial extraction steps.

## General information on sample preparation

The amount of ethanol present in the cuvette should range between 0.25 and 12 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield an ethanol concentration between 2.5 and 120 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.12 to 120 mg/L).

A special attention may be taken to prevent the reaction contamination with unknown sources of ethanol. Thus, all operations should be performed in sealed glass bottles since ethanol is volatile. Pay particular attention to pipetting, diluting and filtering solutions. Plastic tips of dispensing pipettes should be rinsed 3 times with the solution before taking the aliquot. Cuvettes and plastic tips should be rinsed 3 times with ethanol-free distilled water and dried before use. Do not use the pipette used to aliquot the ethanol standard or other ethanol solution, when preparing the assays.

To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 - 9 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 - 9 and incubate for approx. 15 min; measure "coloured" samples (if necessary adjusted to pH 8 - 9) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

## Examples of sample preparation

**Determination of ethanol in white and red wines**

Ethanol concentration in white and red wine can be determined without any sample treatment. Typically, for wines with 10-15 % v/v ethanol, a dilution of 1:1000 and sample volume of 0.1 mL are appropriate.

**Determination of ethanol in dairy products**

Weigh precisely approx. 10 g of sample into a 100 mL bottle and extract with 50 mL of ethanol-free water with agitation for 30 min at 60 °C (keep the bottle sealed). After cooling quantitatively transfer the solution to a 100 mL volumetric flask. Adjust the volume to the mark with ethanol-free water. If required, filter the turbid solutions and dilute. In general, no dilution is required and sample volumes up to 0.5 mL will be appropriated.

**Determination of ethanol in solid foodstuffs**

Solid foodstuffs (~10 g) should be homogenized using a mortar or homogeniser, if necessary. To 2 g of sample add 50 mL of ethanol-free water and stir for 30 min in a sealed bottle (heat at 60 °C, if necessary). Cool the extract, if necessary, and quantitatively transfer to a 100 mL volumetric flask. Dilute to the mark with ethanol-free water. Filter the turbid solution, dilute if necessary and analyse. In general, a dilution of 1:10 and sample volume of 0.1 mL are appropriate.

## References

Beutler, H. O. (1988). Ethanol. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 598-606, VCH Publishers (UK) Ltd., Cambridge, UK.

## Recommendations

This method is recommended/approved by the:

- Austrian, Belgian, German and Swiss food laws;
- French standard (NF);
- International Federation of Fruit Juice Producers (IFU);
- Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.);
- European Brewery Convention (EBC);
- American Society of Brewing Chemists (ASBC);

*Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications*

For life science research only. Not for use in diagnostic procedures.

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