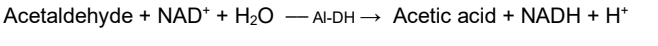
Enzymatic UV assay for the determination of acetaldehyde in foodstuffs and other sample materials
Test combination for 50 determinations

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

This test was validated for the following matrices: Wine, fruit juice, beer, sweet liqueur and yogurt. For detailed results and further information on validation data, please refer to the validation report. Other foodstuffs or sample materials can be tested and must be validated by the user.

1. Test principle

Acetaldehyde is oxidized to acetic acid in the presence of the enzyme aldehyde-dehydrogenase (Al-DH):



The amount of NADH generated in this reaction is stoichiometric to the amount of acetaldehyde. NADH is measured based on its specific absorption at a wavelength of 340 nm.

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 depends on the used device. The incubation times for automated processing may vary and must therefore be validated.

- Reagent 1: 2 x 50 mL with buffer, aldehyde-dehydrogenase
- Reagent 2: 2 x 12.5 mL with buffer, NAD^+

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 indicated shelf life (see labeling) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

This product/test is only suitable for use within the scope of its intended purpose. The instruction for use must be strictly followed.

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

3.1. General

- Sample preparation for manual and automated testing is identical.
- Use liquid, clear and colorless solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Turbid solutions have to be centrifuged or filtered.
- Store samples in a cold and dry room protected from light.

3.1.1 Determination of acetaldehyde in yogurt

Weigh 40 g of the sample to an accuracy of 10 mg into a Falcon tube (50 mL), add 4 mL of 20 % (w/v) citric acid in water, fill the tube to 50 mL with water, close and shake for 5 min on a rotary shaker.

Centrifuge the falcon at 3,000 g for 5 min at 4 °C or transfer a small aliquot of the resulting slurry to an Eppendorf cup and centrifuge at 14,000 rpm at 4 °C for 5 min.

Filter the supernatant through a syringe filter. 1000 µL of the obtained filtrate is used in the test.

3.1.2 Determination of acetaldehyde in red and white wine and juice

Depending on the pH value of the sample, the wine can be used directly or after pH adjustment (sample dilutions have to be considered).
To minimize acetaldehyde losses, it is recommended to measure the sample directly.
Strongly colored red wines should be decolorized with 0.4 g PVPP per 20 mL of red wine; filter before measurement through a syringe filter.
Sample volume: 100 to 200 µL.

3.1.3 Determination of acetaldehyde in sweet liqueur

Pipette 20 mL of the sample into a Falcon tube (50 mL), add the required volume of 1 M NaOH to bring the pH up to 8, add 0.4 g of PVPP, vortex, centrifuge at 3,000 rpm and filter the supernatant using a syringe flter. 500 µL of the obtained solution is used in the test.

3.1.4 Determination of acetaldehyde in beer

Degase beer samples before application in the test by using a syringe filter.

3.2. Important notes

Acetaldehyde is extremely volatile (boiling point approx. 21 °C); therefore, all containers with samples and control solutions must be kept tightly closed.

Because of the high volatility of acetaldehyde, it is always necessary to pipette acetaldehyde-containing solutions below the surface of water or buffer solutions, e.g., when preparing sample or control solutions, especially when diluting these materials, or when dispensing these solutions into the cuvette.

In the presence of oxygen from air acetaldehyde is easily oxidized to acetic acid; therefore, samples must be analyzed as soon as possible after sampling.

A multistepper pipette for adding reagent 1 and reagent 2 is recommended. Use a single tip for each component.

Use separate tips for each sample extract (and control solutions) to avoid cross-contamination, pre-flush the tip before pipetting.

4. Assay procedure

Wavelength: 340 nm
Cuvettes: 1.00 cm light path
Temperature: 20 °C (during the measurement)
Measuring range: 7 - 300 mg/L

Important: Acetaldehyde is very volatile in solution. The sample must be pipetted into reagent 1 below the surface.		
	Reagent blank	**Sample / control**
Reagent 1 **Sample / control** **Dist. water**	2000 µL - 100 µL	2000 µL 100 µL -
Mix, incubate for 3 min at 20 °C*. Read absorbance $\mathbf{A_1}$ at 340 nm, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for 15 min at 20 °C, read absorbance $\mathbf{A_2}$.		

*Alternatively, the measurement can also be carried out at 25 °C or 37 °C. The incubation times do not need to be adjusted in this case.

The reagent blank value (RB) must be determined for each sample and deducted from the respective sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of acetaldehyde

$$\Delta A_{\text{Sample or control}} = (A_2 - A_1 \times df)_{\text{Sample}} - (A_2 - A_1 \times df)_{\text{RB}}$$

df: Dilution factor
RB: Reagent blank

$$df_{100\,\mu L} = \frac{\text{sample volume + volume R1}}{\text{total test volume}} = \mathbf{0.808}$$

Stated df of 0.808 applies for a basic application of 100 µL. Increasing the sample volume is possibly applicable (max. 1000 µL; refer to validation report). With constant reagent volumes conversion of dilution factor (df) is necessary.
If the sample volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$\mathbf{C}_{\text{acetaldehyde}}\ \mathbf{[mg/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v)} = \mathbf{181.8 \times \Delta A} \times F$$

If the sample extract was diluted before measurement, this result has to be multiplied with the pre-dilution factor F.

V: Test volume (basic application) [mL] = 2.600
MW: Molecular weight [g/mol] = 44.05
d: Optical path [cm] = 1.00
v: Sample volume (basic application) [mL] = 0.100
ε: Extinction coefficient NADH [L/mmol x cm] = 6.3 (at 340 nm)

5.2. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. Recovery of aqueous standard solutions should be within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

Other aldehydes (e.g. formaldehyde, propionaldehyde) can be converted by the enzyme if they are present in the food. 5-(Hydroxy-methyl)furfural (HMF) is recovered at almost 100 %.

6.2. Interferences

Sodium sulfite does not interfere with this test at or below 1 g/L.
Ascorbic acid does not interfere at or below 3 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 300 mg/L acetaldehyde, with the recommended measuring range between 7 and 300 mg/L (sample volume of 100 µL) and 0.7 mg/L to 40 mg/L for a sample volume of 1000 µL.

The limit of detection (LoD) was determined according to method DIN 32645:2008-11 in buffered aqueous solution. This results in an LoD of 1.5 mg/L and 0.2 mg/L acetaldehyde for a sample volume of 100 µL and 1000 µL, respectively. The limit of quantification (LoQ) was determined by precision profile and confirms a concentration of 7 mg/L and 0.7 mg/L for 100 µL and 1000 µL sample volume, respectively.

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 under the following link
https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, the individual test procedure and the laboratory environment. Detection and quantification limits depend on the respective sample matrix and the extraction method. For detailed results and further information, please refer to the current validation report.
For the present enzymatic test, only stated, exemplary matrices could be validated due to the large number of foodstuffs and other sample materials.
When analyzing a non-validated matrix, it is recommended to verify the results obtained by means of spike experiments. If necessary, a suitable sample preparation validation for the sample matrix of interest will need to be performed and validated.

9. Services & technical support

On request, we offer the following services:

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

10. Disclaimer

This information corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.