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 (36 – 46 °F)

This test was validated using selected samples of the following matrices: red and white wine, fruit juice, beer, sweet liqueur and yogurt.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

Acetaldehyde is quantitatively oxidized to acetic acid in the presence of aldehyde dehydrogenase (AI-DH) and nicotinamide-adenine dinucleotide (NAD):

Acetaldehyde + NAD^+ + H_2O $\xrightarrow{\text{AI-DH}}$ acetic acid + NADH + H^+

In this process, NAD is reduced to NADH. The amount of NADH formed is stoichiometric to the amount of acetaldehyde. The increase in 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 for automated processing is increased by a multiple; however it depends on the device.

- 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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is identical.
- Bring sample solutions to room temperature before measurement.
- Acetaldehyde evaporates very easily (boiling point approx. 21 °C / 70 °F)! Make sure all containers with samples and control solutions are tightly closed.
- Because of the high volatility, it is highly recommended to pipette acetaldehyde-containing solutions into water or buffer solutions, e.g., when preparing sample or control solutions, especially when diluting these materials.
- In the presence of (atmospheric) oxygen, acetaldehyde is readily oxidized to acetic acid; consequently, samples must be analyzed as soon as possible after sampling.
- Use liquid, clear, colorless and almost neutral sample solutions directly or dilute sufficiently to yield a acetaldehyde concentration within the stated measuring range (refer to performance data).
- In case of higher sample volumes (up to 1000 µL), check the pH value of the test solution and neutralize in case of any doubt.
- Adjust acidic or weakly colored samples to pH 7 – 8 by adding sodium or potassium hydroxide solution. The high volatility of acetaldehyde is of critical significance. In order to avoid financial losses, it is essential to estimate the required volume of hydroxide solution in advance.
- Treat strongly colored samples with activated charcoal or PVPP (e.g. 0.1 g/10 mL up to 0.5 g/10 mL) and filter.
- For turbid samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbonic acid (e.g. beer) by a short burst of ultrasound (ultrasonic bath filled with ice-cold water containing ice cubes), stirring in a beaker, filtration or centrifugation.
- Store samples in a cold and dry room protected from light.

3.2. Determination of acetaldehyde in yogurt

- Weigh 40 g ± 0.010 g of the sample accurately into a reaction or centrifuge tube (50 mL).
- Add 4 mL of 20 % (w/v) citric acid in water.
- Fill up to the mark with distilled water, close and shake for 5 minutes on a rotary shaker.
- Centrifuge the tube at 3000 rpm at 4 °C (39 °F) for 5 minutes. Or, if preferred, transfer a small amount of the resulting slurry to an Eppendorf cup and centrifuge at 14000 rpm at 4 °C (39 °F) for 5 minutes.
- Use a syringe filter to separate the liquid from the solid parts.
- Use 1000 µL of the obtained filtrate in the test.

3.3. Determination of acetaldehyde in red and white wine and juice

- Wine can be used directly or after pH adjustment (sample dilutions have to be considered).
- To reduce the loss of acetaldehyde, it is recommended to measure the samples directly.
- Strongly colored red wines should be decolorized with 0.4 g PVPP to each 20 mL sample.
- Before measurement, filter the wine through a syringe filter.
- Use 100 – 200 µL sample solution in the test.

3.4. Determination of acetaldehyde in sweet liqueur

- Pipette 20 mL of the sample into a 50 mL reaction or centrifuge tube.
- Add the required amount of 1 M NaOH to adjust the pH 8.
- Add 0.4 g of PVPP, mix, and centrifuge at 3000 rpm.
- Filter the supernatant using a syringe filter.
- Use 500 µL of the obtained sample solution in the test.

3.5. Determination of acetaldehyde in beer

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

4. Manual test procedure

Wavelength: 340 nm
Temperature (measurement): 20 – 37 °C (68 – 99 °F)
Photometer alignment: against air (without cuvette)
Measuring range: 7 – 300 mg/L

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for **3 minutes at 20 °C – 37 °C (68 °F – 99 °F)**. **Read** absorbance $\mathbf{A_1}$, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **15 minutes at 20 °C – 37 °C (68 °F – 99 °F)** and **read** absorbance $\mathbf{A_2}$.		

Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at an optimal temperature of 20 °C (68 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**, without having to adjust the incubation times.
  It is important to note that incubation at 37 °C (99 °F) may result in a slight decrease in the reaction curve, leading to reduced recoveries of acetaldehyde.
- Acetaldehyde exhibits a high degree of volatility in solution. **Samples are required to be pipetted into reagent 1 beneath the surface**.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 2- or 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Concentration of acetaldehyde in sample solutions

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume [mL]} + \text{volume R1 [mL]}}{\text{reaction volume [mL]}} = 0.808$$

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df) accordingly**.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of acetaldehyde in sample solutions is calculated using Lambert-Beer's law:

$$\mathbf{C}_{\text{acetaldehyde}}\ \mathbf{[mg/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{181.8 \times \Delta A} \times F$$

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

V:	Test volume (basic application) [mL]	= 2.600
MW:	Molecular weight acetaldehyde [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. Content of acetaldehyde in solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. For this purpose, we recommend certified reference material, for example:

- Acetaldehyde solution, CRM868095, Merck, 100 x 1.2 mL ampoules, concentration: 1000 µg/mL in water; expanded uncertainty: 70 mg/L (k = 2).

After opening the ampoule, store it in an ice bath, sealed with parafilm. The recovery of aqueous control solutions and extracted food samples should be 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

Compounds tested for side activity were formaldehyde, propionaldehyde, crotonaldehyde, glycolaldehyde, benzaldehyde, glyceraldehyde, glutaraldehyde and hydroxymethylfurfural.

All of these substances exhibited side activity, with benzaldehyde demonstrating the lowest activity, glycolaldehyde exhibiting a quantitative turnover, and 5-(hydroxymethyl)furfural (HMF) exhibiting a side activity of almost 100 %.

6.2. Interferences

Sodium sulfite, ascorbic acid, ethanol, and mixtures of sugars and organic acids were examined for interference in the presence of acetaldehyde. The presence of sodium sulfite and ascorbic acid at concentrations of 1 g/L and 3 g/L, respectively, did not result in interference.

The presence of ethanol and mixtures of organic acids and sugars did not result in interference at or below 50 % and 5 or 10 g/L, respectively.

6.3. Linearity, measuring range & sensitivity

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

The limit of detection (LoD) was determined according to the method DIN 32645:2008-11 in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 1.5 mg/L and 0.2 mg/L for a sample volume of 1000 µL.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 7.0 mg/L acetaldehyde for a sample volume of 100 µL and 0.7 mg/L for a sample volume of 1000 µL.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Acetaldehyde Validation reports
- Enzytec™ *Liquid* Acetaldehyde Excel template for results
- Enzytec™ *Liquid* Acetaldehyde Technical Information
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

On request, we offer the following services:

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

10. Disclaimer

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