UV assay for the determination of acetic acid 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 evaluated using selected samples of the following matrices: wines, juices, sauces/remoulades, kombucha, beer, sausages/meat, vinegar, and microbiological culture media.

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

The test has been approved as AOAC *Official Method of Analysis* 2024.01 First Action. A publication is available in J. AOAC Int. 108(3), 395–411 (2025).

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

Acetic acid (acetate) is converted by adenosine 5'-triphosphate (ATP) into acetyl phosphate and adenosine 5'-diphosphate (ADP) in the presence of the enzyme acetate kinase (AK). The amount of ADP formed is equimolar to the acetate concentration and is the limiting factor for the following steps:

Acetate + ATP $\xrightarrow{AK}$ acetylphosphate + ADP

For every mole of ADP present in the reaction, one mole of D-glucose is converted by an ADP-dependent hexokinase into D-glucose-6-phosphate (G-6-P) and adenosine-5'-monophosphate (AMP):

ADP + D-glucose $\xrightarrow{ADP\text{-}HK}$ G-6-P + AMP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P and the coenzyme nicotinamide adenine dinucleotide (NAD) react to form D-glucono-δ-lactone-6-phosphate (GdL-6-P):

G-6-P + NAD^+ $\xrightarrow{G6P\text{-}DH}$ GdL-6-P + NADH + H^+

NAD is reduced to NADH in the process. The amount of NADH formed is measured at 340 nm.

Since there is no linear relationship between the acetic acid concentration and the absorbance A measured at 340 nm, four calibrators with defined concentrations are included with the test kit.

A two-point calibration leads to inaccurate results across the entire calibration range.

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, NAD, ATP
- Reagent 2: 2 x 12.5 mL with buffer, AK, ADP-HK, G6P-DH
- Calibrator-Set: 4 x 3.5 mL (20, 100, 300 and 1300 mg/L acetate)

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 the same.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- Samples solutions should be brought to room temperature before measurement.
- Neutralize **strongly** acidic samples by adding 1 M KOH to a pH of 6.5 – 7.5.
- For turbid test samples (e.g., apple cider vinegar, juices): filter the test solution through a pleated paper or syringe filter, or centrifuge it in a test tube (3000 rpm for at least 5 minutes is recommended) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide (e.g., beer) by aid of a short ultrasound burst (10s) or by stirring in a beaker.
- If necessary, decolorize **strongly** colored samples with polyvinylpolypyrrolidone (PVPP).
- Crush and homogenize solid and semi-solid samples and extract with water. Filter or centrifuge afterwards.
- For samples with a high fat content, weigh e.g. 5 g into a 100 mL volumetric flask, fill halfway with water, and heat in a water bath at 50 – 60 °C (122 – 140 °F) for 20 minutes. After cooling, fill the flask to the mark and place it in the refrigerator for about 20 minutes to separate the fat. Then use a pleated filter to obtain a clear or slightly cloudy sample (discard the first few milliliters).
- In case of higher sample volumes (up to 1000µL), check the pH value of the test solution and neutralize in case of any doubt.

3.2. Juices and wines

- For juices and wines used undiluted, add 0.1 g of PVPP per 10 mL of sample and stir or shake for 1 minute.
- Then filter (using a paper or syringe filter) or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- For highly colored samples, the amount of PVPP can be increased to up to 0.5 g.

3.3. Sauces

- Weigh 10 g of the sample into a beaker (e.g., 150 mL), add 50 mL of water, and stir on a magnetic stirrer for 10 minutes. If necessary, heat the sample to 60 °C (140 °F) while stirring.
- Transfer the suspension quantitatively to a 100 mL volumetric flask, allow the test solution to cool, and dilute with water to the mark.
- Transfer 10 mL of this suspension to a second 100 mL volumetric flask and dilute with water to the mark. After shaking, use a pleated filter to obtain a clear test solution (discard the first approximately 15 mL) or use a syringe filter.

3.4. Heated meat products

- Accurately weigh approximately 10 g of a well-homogenized sample into a resealable 50 mL plastic tube and add 20 mL of distilled water.
- Mix vigorously using a vortex mixer, bring the volume up to 50 mL with distilled water, and heat in a water bath at 70 °C (158 °F) for 15 minutes.
- Add one drop of 98 % sulfuric acid and transfer the suspension quantitatively with water into a 100 mL volumetric flask.

- Allow the test solution to cool to room temperature and fill the volumetric flask with distilled water until the meniscus of the aqueous layer reaches the 100 mL mark (oil phase above the 100 mL calibration mark).
- Mix carefully by rotating and swirling the volumetric flask, then filter (using a paper or syringe filter) and use the filtrate for analysis (directly or after dilution within the measurement range).

3.5. Raw meat products

- When analyzing raw meat products, creep reactions may occur due to the interfering effects of enzymes and substrates in the raw material.
- To prevent these creep reactions, heat 50 to 100 g of the sample to 75 °C (167 °F) for 15 minutes before homogenization, and then follow the instructions described above for heated meat products.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	20 – 1300 mg/L (for 100 µL)

	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 – 37 °C (68 – 99 °F)**. **Read absorbance A_1**, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **15 minutes at 20 – 37 °C (68 – 99 °F)** and **read absorbance A_2**.		

4.1. 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 and from each calibrator**.
- Specified incubation times were validated at 25 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- The calibration curve should be visually checked to ensure that the values increase steadily. The first term of the third-degree polynomial equation should be positive. The measured absorbance difference of the samples must lie within the calibration curve.
- If the measured absorbance difference of the samples is too small, the sample solution must be prepared again using a higher initial weight or a less diluted solution.
- If the absorbance difference of the samples is very large, the sample solution must be diluted if necessary.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- The use of a multistepper pipette is recommended for adding the 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

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of acetic acid

The extinction difference ΔA must be calculated for each sample:

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

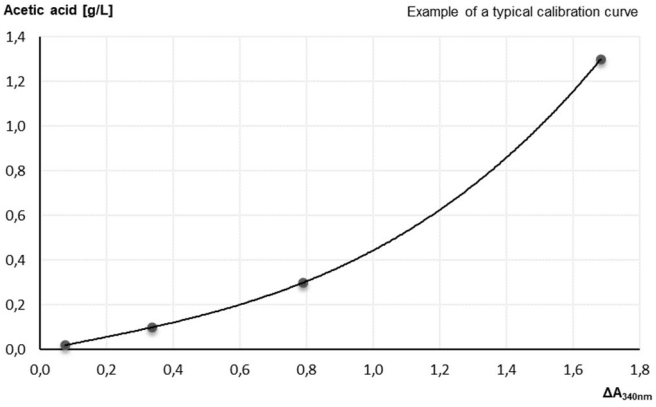
df: Dilution factor
RB: Reagent blank

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

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 calibration curve is determined in Excel using a 3rd degree polynomial. The target concentration values of the calibrators are plotted against the corresponding ΔA values. The concentration of the samples is determined using the polynomial equation. An Excel template for calculation is available on request.



Example of a calibration curve using a third-order polynomial curve fitting; concentration values are plotted against the corresponding ΔA values.

5.2. Calculation of 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:

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

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ Liquid Multi-Acid Standard low (Art. No. E8460).

The recovery of this multi-standard low and other aqueous control solutions should be 100 ± 5 %.

As a certified (standard) reference material, we recommend:

- Acetic acid Standard solution, HACH (Art. No. 1420542)

5.4. Calibration

The calibration stability is 7 days. The validity of the calibration should be verified daily with a control sample.

To ensure the validity of the calibration over the specified period (7 days), aqueous control solutions should be analyzed during each run. If these control solutions do not fall within the specifications, recalibration must be performed.

6. Performance data

6.1. Specificity & side activities

The test is specific for acetate and shows no relevant side-reactivity with related substances.

Highly concentrated organic acids were tested for cross-reactivity in the assay system, including sorbic acid (1 g/L), fumaric acid (5 g/L), ethyl acetate, benzoic acid, butyric acid, citric acid, hydroxybutyric acid, D- and L-lactic acid, D- and L-malic acid, formic acid, L-ascorbic acid, L-tartaric acid, oxalacetic acid, oxalic acid, propionic acid, salicylic acid, shikimic acid, and succinic acid (10 g/L each).

Only D-lactic acid, fumaric acid, oxalacetic acid, and propionic acid exhibited measurement signals other than zero. However, these were below the absorbance of standard 1 (20 mg/L) and irrelevant.

6.2. Interferences

Major organic acids were tested for their interfering effects in the presence of 1 g/L acetic acid, including D- and L-malic acid, formic acid, L-ascorbic acid, benzoic acid, succinic acid, butyric acid, citric acid, ethyl acetate of acetic acid, fumaric acid, hydroxybutyric acid, D- and L-lactic acid, oxalacetic acid, oxalic acid, propionic acid, salicylic acid, shikimic acid, sorbic acid, and L-tartaric acid. Due to limited solubility or naturally occurring concentrations, different concentrations in the range of 0.5 g/L to 3 g/L were selected. All these substances showed no interferences.

Even high concentrations of D-glucose and D-fructose (150 g/L each), glycerol (25 g/L), and SO_2 (1 g/L) showed no interference in the presence of 1 g/L acetic acid.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1500 mg/L acetic acid (sample volume of 100 µL) with a recommended measuring range of 20 – 1300 mg/L.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the determined LoD is 2.2 mg/L. The limit of quantification (LoQ) is of 3.8 mg/L for a sample volume of 100 µL.

The smallest absorbance difference reliably distinguishable by the method is $\Delta A = 0.005$. By increasing the sample volume, the sensitivity of the assay can be improved accordingly.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	90 mg/L
Basic Range	16 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	up to 6.5 g/L
Basic Range	up to 1300 mg/L

6.4.3. Precision and accuracy

Data from the measurement of an aqueous solution are shown here.

High Range

Target concentration, mg/L	150	1008
Mean value, mg/L	153.1	992
SD, mg/L	5.10	10.4
RSD, %	3.3	1.0
Recovery, %	102.1	98.4

Basic Range

Target concentration, mg/L	150	994
Mean value, mg/L	152.4	1009
SD, mg/L	1.60	13.4
RSD, %	1.1	1.3
Recovery, %	101.6	100.1

7. Supporting documents

On request, we offer the following documents:

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