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

1. Test principle

Enzymatic test with acetate kinase (AK), ADP hexokinase (ADP-HK) and glucose-6-phosphate-dehydrogenase (G6P-DH).

Acetic acid (acetate) reacts in the presence of ATP and acetate kinase (AK) to acetylphosphate and ADP. The amount of ADP formed is equimolar to the concentration of acetate and the limiting factor for the following steps:

Acetate + ATP —— AK ⟶ acetylphosphate + ADP

For each mol of ADP present in the reaction, one mol of D-glucose is converted by an ADP-dependent hexokinase into D-glucose-6-phosphate and AMP.

ADP + D-glucose — ADP-HK ⟶ D-glucose-6-phosphate + AMP

In the presence of glucose-6-phophate-dehydrogenase (G6P-DH, D-glucose-6-phosphate and nicotinamide-adenine-dinucleotide (NAD^+) react to D-glucono-δ-lactone-6-phosphate and $NADH/H^+$.

D-glucose-6-phosphate + NAD^+ — G6P-DH ⟶
D-glucono-δ-lactone-6-phosphate + NADH + H^+

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

Since there is no linear relationship between the acetic acid concentration and the OD measured at 340 nm, four calibrators are included in the test kit. A two-point calibration as used by some competitors does not result in accurate results over the whole 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 acetic acid)

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).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Clarify samples containing proteins or fat with Carrez reagents.
- Crush and homogenize solid and semi-solid samples, extract suitable sample amount with water.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 20 - 1300 mg/L

	Reagent blank	**Samples / controls**
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. Concentration of acetic acid

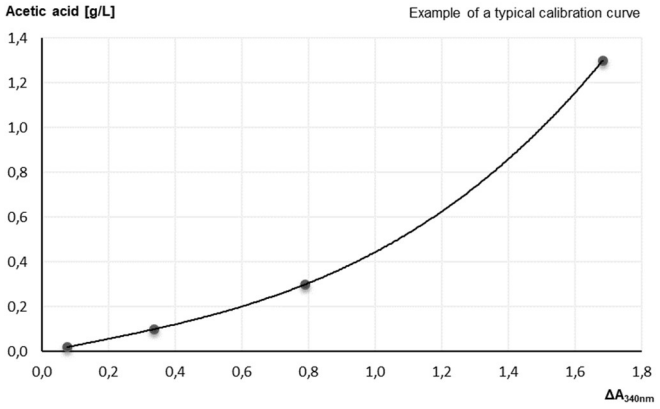
$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

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.

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.



5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-Acid Standard low (E8460).
The recovery of Enzytec™ *Liquid* Multi-Acid Standard low and other aqueous control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity & side activities

The determination is specific for acetic acid. For the determination of side activities of the measurement system, high concentrated organic acid solutions (10 g/L) were measured in the acetic acid reagent. D-lactic acid, fumaric acid, oxaloacetic acid and propionic acid do not show any side activities.

6.2. Interferences

All following substances have been tested at higher individual concentrations in the presence of 1 g/L acetic acid and showed no interferences (for details see validation report).

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1500 mg/L acetic acid, with the recommended measuring range between 20 and 1300 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using buffered aqueous solution. This results in an LoD of 2.2 mg/L. The limit of quantification (LoQ) is of 3.8 mg/L.

6.4. Calibration

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

6.5. Automation with Pictus 500

6.5.1. Limit of quantification (LoQ)

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

6.5.2. Measuring ranges

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

6.5.3. 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* 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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