Enzymatic assay for the determination of acetic acid in foodstuff and other sample materials
2 x 50 mL R1 and 2 x 12.5 mL R2 – 50 assays (manual) / ≥ 500 assays (auto-analyzer)

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

Principle

Enzymatic test with Acetate kinase (AK), ADP-Hexokinase (ADP-HK) and Glucose-6-phosphate dehydrogenase (G6P-DH). NADH is produced and measured at 340 nm:

Acetate + ATP —— AK ⟶ Acetyl phosphate + ADP

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

D-Glucose-6-phosphate + NAD^+ — G6P-DH ⟶
6-Phosphoglucono-δ-lactone + NADH + H^+

Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (Buffer, NAD, ATP)
- Reagent 2: 2 x 12.5 mL (AK, ADP-HK, G6P-DH)
- Calibrator-Set: 4 x per 3.5 mL (20, 100, 300 and 1300 mg/L acetic acid)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

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 material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Use liquid, clear and almost neutral samples directly or after dilution with dist. water to a concentration within the measuring range (see performance data) in the test (consider dilution factor in calculation).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon 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 for fat separation; fill volumetric flask up to the mark with water, filter aqueous solution before testing.
- Detailed sample preparation guideline available on request.

Assay procedure

Wavelength: 340 nm
Optical path: 1 cm
Temperature: 37 °C or 20 - 25 °C
Measurement: against air or against water
Sample solution: 20 to 1300 mg/L

	Reagent blank (RB)	**Samples / calibrators**
Reagent 1	2000 µL	2000 µL
Sample / calibrator	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 37 °C or at 20 - 25 °C. Read absorbance A_1 **in time** as possible, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate 10 min at 37 °C or 15 min at 20 - 25 °C. Read absorbance A_2 **in time** (no endpoint determination).		

The reagent blank value must be determined once for each run and subtracted from each sample result.

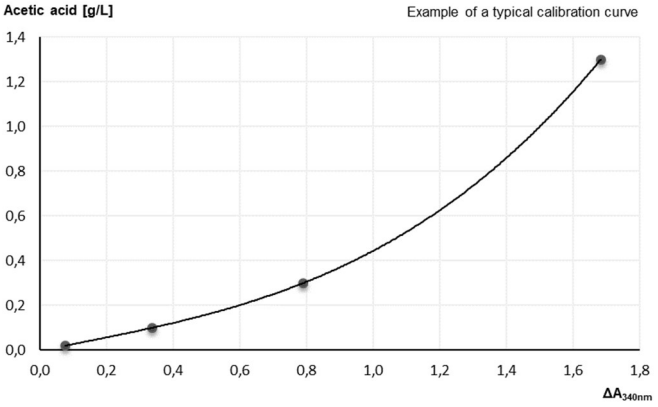
Calculation of results

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 or directly from the graph. An Excel template for calculation is available on request.

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

df = (Reagent) dilution factor; RB = Reagent blank

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



Calculation for solid samples:

$$Content_{acetic\ acid}\ [g/100\ g] = \frac{C_{acetic\ acid}\ [g/L]}{weight_{sample}\ [g/L]} \times 100$$

Performance data

Specificity

The determination is specific for acetic acid. Interferences were measured for ascorbic acid up to 1.0 g/L, for citric acid up to 2.5 g/L, for tartaric acid up to 3.5 g/L, for glycerol up to 25 g/L and for sulphite (SO_2) up to 1 g/L and can be excluded.

Measuring range & calibration

The recommended measuring range is 20 to 1300 mg/L.
The calibration stability is 7 days. The validity of the calibration should be verified daily with a control sample.

Sensitivity

The limit of detection (LoD) and the limit of quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution for a sample volume of v = 100 µL.
This results in an LoD of 2.5 mg/L and an LoQ of 4.5 mg/L.

Automation & validation report

Application sheets for automated systems and customer validation reports are available on request.

Disclaimer

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