Enzymatic assay for the determination of citric 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

Method

Enzymatic UV determination of citric acid with with citrate lyase (CL), L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH).

Principle

Citric acid (citrate) is cleaved into oxaloacetate and acetate in the presence of the enzyme CL:

Citric acid $\xrightarrow{CL}$ oxalacetate + acetate

The resulting oxaloacetate and its decarboxylation product pyruvate are reduced to L-malate and L-lactate respectively:

Oxalacetate + NADH + H^+ $\xrightarrow{L\text{-}MDH}$ L-malate + NAD^+

Pyruvate + NADH + H^+ $\xrightarrow{L\text{-}LDH}$ L-lactate + NAD^+

Reduced nicotinamide adenine dinucleotide (NADH) is oxidized to NAD. The amount of NADH consumed is equivalent to the amount of citric acid converted and is measured at a wavelength of 340 nm.

Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (NADH, L-MDH, L-LDH)
- Reagent 2: 2 x 12.5 mL (buffer, CL)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C (see label). 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 nearly neutral samples directly or after dilution into the relevant measuring range (see test performance).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- If necessary, decolorize strongly colored samples with PVPP.
- Crush and homogenize solid and semi-solid samples, weigh in a suitable sample amount and extract with water.
- For clarification of protein-containing samples, preparation with perchloric acid or trichloroacetic acid is recommended.
- Carrez clarification is unsuitable, as this absorbs citric acid!
- Detailed sample preparation guideline available on request.

Assay procedure

Wavelength: 340 nm
Temperature: 37 °C or 20 - 25 °C
Measurement: against air or against water
Sample: 25 - 1000 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 add:		
Reagent 2	500 µL	500 µL
Mix, wait for the end of the reaction (incubate for about 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.

Calculation of results

Calculation of sample solutions:

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

df: dilution factor
RB: Reagent blank

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

$$c_{Citric\ acid}\ [g/L] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)}$$

V:	Test volume [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 192.13
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

For a determination at 340 nm this results in:

$$c_{Citric\ acid}\ [g/L] = 0.7929 \times \Delta A$$

Calculation of solid samples:

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

Performance data

Specificity

The test is specific for citric acid and shows no side activities or interferences with other relevant acids. Sulfite and meso-tartaric acid do not interfere at or below 3.13 g/L.

Linearity & Measuring range

Linearity is given up to 1400 mg/L citric acid. The recommended measuring range is between 25 and 1000 mg/L citric acid.
If this range is exceeded, the samples should be diluted with dist. water to an ammonia concentration within the measuring range. The dilution factor must be taken into account in the calculation.

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:

- Sample volume v = 100 µL: LoD = 15 mg/L, LoQ = 25 mg/L
- Sample volume v = 1000 µL*: LoD = 2 mg/L, LoQ = 3 mg/L

*increased sample volume requires pH neutralization

Automation & Validation report

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

Disclaimer

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