UV assay for the determination of citric 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

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

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

The resulting oxaloacetate and its decarboxylation product pyruvate are reduced (in the presence of L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH)) 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.

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, NADH, L-MDH, L-LDH
- Reagent 2: 2 x 12.5 mL with buffer, CL

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.
- Degas samples containing carbonic acid.
- 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.
- If necessary, decolorize strongly colored samples with PVPP.
- For clarification of protein-containing samples, preparation with perchloric acid or trichloroacetic acid is recommended.
- Carrez clarification is unsuitable, as this absorbs citric acid!

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 40 - 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 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 citric acid

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

df: Dilution factor
RB: Reagent blank

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

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume of very acidic samples is increased, the test system may be affected. This must be checked.

$$\mathbf{C_{Citric\ acid}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.7929 \times \Delta A}$$

V:	Test volume basic application [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)

5.2. Calculation of solid samples

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

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 % and for extracted food samples within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

The test is specific for citric acid and shows no side activities with other relevant acids.

6.2. Interferences

Sulfite and meso-tartaric acid do not interfere at or below 3.13 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1400 mg/L citric acid, with the recommended measuring range between 40 and 1000 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 in buffered aqueous solution. This results in an LoD of 15 mg/L. The limit of quantification (LoQ) was determined by precision profile and is 40 mg/L.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an calculated LoD of 0.53 mg/L. Based on ΔA = 0.010, an LoQ of 1.06 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	0.5 g/L
Basic Range	0.04 g/L
Sensitive Range	8 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	to 5 g/L
Basic Range	to 1 g/L
Sensitive Range	to 100 mg/L

6.4.3. Precision and accuracy

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

High Range

Target concentration, g/L	1.0	0.25
Mean value, g/L	0.989	0.248
SD g/L	0.0104	0.0048
RSD, %	1.05	1.93
Recovery, %	98.9	99.1

Basic Range

Target concentration, g/L	1	0.5	0.25
Mean value, g/L	1.012	0.494	0.252
SD g/L	0.0061	0.0027	0.0028
RSD, %	0.60	0.54	1.13
Recovery, %	101.2	98.8	100.6

Sensitive Range

Target concentration, g/L	0.1	0.05	0.025
Mean value, g/L	0.100	0.048	0.024
SD g/L	0.0009	0.0007	0.0012
RSD, %	0.94	1.54	5.17
Recovery, %	100.0	95.8	95.2

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 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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