Enzymatic determination of citric acid in foodstuff and other sample materials
Test-kit for 32 determinations on the RIDA®CUBE SCAN instrument (340 nm)

For in vitro use only
Store between +2 and +8 °C

Principle

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

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

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:

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

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

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

1: 32 tubes with approx. 800 µL reagent 1 (buffer)

2: 32 caps with approx. 200 µL reagent 2 (enzyme)

3: one RFID-card (Radio Frequency Identification)

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 on our website (www.r-biopharm.com). After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

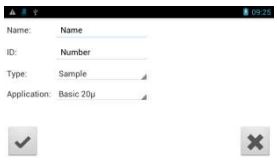
- Use clear, colorless and pH-neutral sample solutions directly or after dilution in the relevant measuring range (see test results)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Clarify samples containing protein and fat with perchloric acid or trichloroacetic acid
- Crush and homogenize solid and semi-solid samples and extract with water; filter or centrifuge, or use a clarification method with acids if necessary
- Weigh samples with a high fat content into a volumetric flask (min. 50 mL) and extract with hot water; allow the sample solution to cool for fat separation (e.g. 15 min in an ice bath); fill the volumetric flask up to the mark with water, remove the fat layer and filter the aqueous solution before testing
- Adjust strongly alkaline or strongly acidic samples to approx. pH 8 with KOH / NaOH or HCl

Assay specifications

The assay specifications are saved on the RFID card and are executed automatically by the instrument.

Wavelength:	340 nm
Temperature:	37 °C
Calibration:	calibration curve saved on RFID card
Test sequence:	sample + R1 / mix / 2 min / A1 / R2 / mix / 15 min / A2
Sample volume:	20 µL (Basic) or 100 µL (Sensitive). The required volume should be pipetted precisely into the test tube (reagent 1).

Handling procedure

Place the RFID-card on the instrument	
Enter sample data into tablet app : - identification - volume (20 or 100 µL)	
Pipette the sample into the test-tube (reagent 1): - 20 or 100 µL	 
Close the tube with the cap (reagent 2), insert into the instrument and close the door	 

Test performance

Measuring range

The results are given in mg/L by the instrument, and following ranges are recommended:
- from 60 to 1000 mg/L for the basic application (20 µL)
- from 20 to 225 mg/L for the sensitive application (100 µL)

Notes

1. Use a quality control every day where a run is performed (e.g. Enzytec™ *Liquid* Multi-Acid Standard Low, Art. No. E8460). If the deviation of this quality control is higher than 10%, it is necessary to measure the reagent blank with a water sample, and to subtract it from all future samples results.