Enzymatic assay for the determination of D-*threo*-isocitric 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 D-*threo*-isocitric acid with isocitrate dehydrogenase (IC-DH).

Principle

D-*threo*-isocitric acid (D-*threo*-isocitrate) is converted to oxoglutarate by nicotinamide adenine dinucleotide phosphate (NADP) in the presence of the enzyme isocitrate dehydrogenase (IC-DH):

D-isocitrate + $NADP^+$ —IC-DH→ 2-oxoglutarate + CO_2 + NADPH + H^+

In this process, NADP is reduced to NADPH. The amount of NADPH formed in this reaction is equivalent to the converted amount of D-*threo*-isocitric acid and is measured at a wavelength of 340 nm.

Bound D-isocitric acid is determined after alkaline hydrolysis according to the same principle.

D-isocitric acid ester + H_2O —pH 9-10→ D-isocitrate + alcohol

D-isocitric acid lactone + H_2O —pH 9-10→ D-isocitrate

Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (buffer, NADP)
- Reagent 2: 2 x 12.5 mL (buffer, IC-DH)

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 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.
- Crush and homogenize solid and semi-solid samples, weigh in a suitable sample amount and extract with water.
- Strongly colored juices or wines applied undiluted: neutralize 25 mL sample with 2 M NaOH to pH 7 - 7.5, fill up to 50 mL, add 0.5 g PVPP, then filter or centrifuge.
- 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: 6 - 1500 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 37 °C or at 20 - 25 °C. Read absorbance A_1, then add:		
Reagent 2	500 µL	500 µL
Mix, wait for the end of the reaction (incubate for 15 min at 37 °C or at 20 - 25 °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:

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

df: (reagent) dilution factor
RB: Reagent blank

$$df_{basic\ application} = \frac{sample\ volume + R1}{test\ volume} = 0.808$$

Increasing the sample volume (up to 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor.

$$c_{D\text{-}threo\text{-}isocitrate}\ [g/L] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)}$$

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)

For a determination at 340 nm this results in:

$$c_{D\text{-}threo\text{-}isocitrate}\ [g/L] = 0.7929 \times \Delta A$$

Calculation of solid samples:

$$Content_{D\text{-}threo\text{-}isocitrate}\ [g/100\ g] = \frac{c_{D\text{-}threo\text{-}isocitrate}\ [g/L]}{weight_{sample}\ [g/L]} \times 100$$

Performance data

Specificity

The test is specific for D-*threo*-isocitric acid and shows no side activities or interferences with other relevant acids. SO_2 interferes at concentrations below 50 mg/L.

Linearity & Measuring range

Linearity is given up to 1900 mg/L D-*threo*-isocitrate. The recommended measuring range is between 6 and 1500 mg/L D-*threo*-isocitrate. If this range is exceeded, the samples should be diluted with dist. water to a concentration within the measuring range.
The dilution factor must be taken into account in the calculation.

Sensitivity

The lower 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 = 1.0 mg/L, LoQ = 6.0 mg/L
- Sample volume v = 1000 µL: LoD = 0.15 mg/L, LoQ = 0.40 mg/L

Automation & Validation reports

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

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