Enzymatic assay for the determination of D-lactic 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 test for the quantitative determination of D-lactic acid with D-lactate dehydrogenase (D-LDH). For the determination of the sum of D- and L-lactic acid, the test Enzytec™ *Liquid* D-/L-Lactic acid E8240 is suitable. If only the concentration of L-lactic acid is required, use Enzytec™ *Liquid* L-Lactic acid E8260 or subtract the determined D-lactic acid concentration from the result of the test E8240.

Principle

D-lactic acid is converted to pyruvate in the presence of the enzyme D-LDH:

D-lactic acid + NAD^+ $\xrightarrow{\text{D-LDH}}$ pyruvate + NADH + H^+

Nicotinamide adenine dinucleotide (NAD) is reduced to NADH in the process. The consumed amount of NAD is equivalent to the converted amount of D-lactic acid and is measured at 340 nm.

Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (buffer, D-LDH)
- Reagent 2: 2 x 12.5 mL (buffer, NAD)

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.
- Crush and homogenize solid samples, weigh out appropriate sample amount and extract with water.
- Clarify protein-containing samples with Carrez reagents.
- Extract samples with a high fat content with hot water, then allow to cool (ice or refrigerator) for fat separation. Remove fat layer and filter aqueous solution.
- 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: 20 - 500 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 about 10 min at 37°C or 15 min 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: dilution factor
RB: Reagent blank

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

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

V:	Test volume [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 90.08
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:

$$\mathbf{c_{D\text{-lactic acid}}\,[g/L] = 0.3718 \times \Delta A}$$

Calculation of solid samples:

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

Performance data

Specificity

The test is specific for D-lactic acid and shows no side activities or interferences with other relevant organic acids up to 20 g/L. Sulfite and ascorbic acid showed no interference at or below a concentration of 0.02 g/L.

Linearity & Measuring range

Linearity is given up to 700 mg/L D-lactic acid, with the recommended measuring range being between 20 and 500 mg/L.
If this range is exceeded, the samples should be diluted with dist. water to a D-lactic acid 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 for a sample volume of v = 100 µL.
This results in an LoD of 5 mg/L and an LoQ of 20 mg/L.

For a maximum sample volume of v = 1000 µL and a test volume of V = 3.5 mL, theoretical LoD and LoQ values were determined by calculation according to Lambert-Beer.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005, resulting in an LoD of 0.21 mg/L.
Based on ΔA = 0.010, an LoQ of 0.43 mg/L was calculated.

Automation

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

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