Enzymatic assay for D- and L-Lactic acid in foodstuff and other sample materials (without differentiation)
2 x 50 ml R1 and 2 x 12.5 ml R2 (50 assays)

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

Principle

Enzymatic test with D-Lactate dehydrogenase (D-LDH) and L-Lactate dehydrogenase (L-LDH), without differentiation. NADH is produced and is measured at 340 nm:

D-/L-Lactate + NAD^+ —— D-LDH/L-LDH ⟶ Pyruvate + NADH + H^+

Reagents

The reagents are ready-to-use.
Reagent 1: two vials ≥ 50 ml (buffer, D-LDH, L-LDH)
Reagent 2: two vials ≥ 12.5 ml (NAD)

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 further 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 clear, colourless and pH-neutral liquid samples directly, or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Clarify samples containing proteins or fat with Carrez clarification
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge, or use Carrez clarification if necessary
- For fat containing samples, weigh sample into a volumetric flask (min. 50 ml) and extract with hot water; cool to allow the fat to separate; make up to the mark with water, remove the fatty layer on the top and filter the aqueous part
- Adjust the pH to approx. 8.0 by adding KOH / NaOH to acidic samples or by adding HCl to alkaline samples

Assay procedure

Wavelength: 340 nm
Optical path: 1 cm
Temperature: 20 – 25 °C / 37 °C
Measurement: Against air or against water
Sample solution: 10 – 500 mg/l

	Reagent Blank (RB)	Samples
Sample / Standard	-	100 µl
Dist. water	100 µl	-
Reagent 1	2000 µl	2000 µl
Mix, incubate for 1 min. at 37 °C or 3 min. at 20 - 25 °C. Read absorbance A1 , then add:		
Reagent 2	500 µl	500 µl
Mix, wait until the end of the reaction (incubation for approx. 10 min. at 37°C or approx. 15 min. at 20 - 25 °C). Read absorbance A2.		

Reagent blank must be performed once for every run, and subtracted from every sample during calculation of results.

Calculation of results

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
With df = dilution factor of optical densities, because of reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.808.

c = (V x MW x Δ A) / (ε x d x v x 1000) [in g/l of D-/L-Lactate]
c = (2.600 x 90.1 x Δ A) / (ε x 1 x 0.1 x 1000)
It results for the determination at 340 nm (ε = 6.3 $l.mmol^{-1}.cm^{-1}$):

$C_{D-/L-Lactate}$ [g/l] = **0.3718** x ΔA

Calculation in solid samples:

$$Content_{Analyte}\ [g/100\ g] = \frac{C_{Analyte}\ [g/l]}{weight_{sample}\ [g/l]} \times 100$$

Test performance

Specificity

The test is specific for D- and L-Lactic acid. Interferences were measured for Ascorbic acid, Hydroxybutyric acid and Sulfite (SO_2) starting from 0.02 g/l. Oxalic acid interfered above 0.2 g/l, and all other measured substances did not interfere up to 20 g/l.

Linearity and measuring range

The test is linear up to 500 mg/l D-/L- Lactic acid. The recommended measuring range lies between 25 and 500 mg/l, in order to keep ΔA ≅ 1.5 (A). When values exceed this range, samples should be diluted into the range 50 to 500 mg/l with dist. water. The dilution factor has to be considered in the calculation.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

- LoD = 5 mg/l
- LoQ = 10 mg/l

Automation

Application sheets for automated systems are available on request.

Disclaimer

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