

D – LACTIC ACID

UV DETERMINATION IN WINE, FOOD & BEVERAGES

Kit: 5 x 20 mL

Code LA9912

PRINCIPLE

The D-Lactic acid is changed in pyruvate by D-LDH (lactate dehydrogenase) in the presence of NAD. This reaction is helped by a secondary one, who transforms the pyruvate originated before in the presence of glutamate and GPT (glutamate-pyruvate transaminase). The intensity of the UV-colour at this wavelength is proportional to the concentration of D-Lactic acid in the tested sample.

REAGENTS

Components of the kit:

*REAGENT 1 (buffer, liquid, ready to use)

*REAGENT 2 (lyo)

*REAGENT 3 (starter, liquid, ready to use)

Good buffer > 20 mmol/L

Glutamic ac. > 3 mmol/L

NAD > 0.4 mmol/L

LDH > 300 U/L

GPT(ALAT) > 10 U/L

Code LA9912

2 x 100 mL

5 x 20 mL

1 x 2.5 mL

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

PREPARATION OF THE WORKING REAGENT

Dissolve a vial of *Reagent 2 with 20 mL of *Reagent 1 and mix gently till dissolution. Please avoid foaming.

A suggestion could be to aliquot in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagents reach the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

STABILITY: the diluted *Reagent 2 is stable 1 days at 2-8°C.

Till 30 days frozen at -20°C. FREEZE only ONE TIME.

DO NOT REPEAT FREEZING.

AUXILIARY REAGENTS FOR CALIBRATION and for QUALITY CONTROL (Not supplied with the kit)

We suggest strongly to calibrate always on the instruments.

To grant a good calibration we suggest to use following kit:

- SUBSTRATE ELEVATED CONTROL Cod. SCE 3005

To grant the correct test performances we suggest to use following kits:

- SUBSTRATE LOW CONTROL Cod. SCL 3006

- SUBSTRATE ELEVATED CONTROL Cod. SCE 3005

Use them as the samples in the following PROCEDURES.

SAMPLE

- Wine could be used directly.
- Use colourless, clear and quite neutral liquid samples directly if D-Lactic acid conc. is between 0.020–0.500 g/L; otherwise, dilute with water to reduce it in this range.
- Turbid solutions have to be filtered or centrifuged
- Samples containing carbon dioxide have to be degassed.
- Acid samples have to be adjusted by adding KOH /NaOH until approx. pH 8 is reached.
- Alkaline samples have to be adjusted by adding HCl until approx. pH 8 is reached.
- Strongly coloured samples have to be treated with PVPP (polyvinylpyrrolidone e.g. 1 g/100 mL Sample).
- For other different samples, please inquire the use and for potential pre-treatment.

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37°C
- Method: end-point
- Reaction: 10 - 25 minutes
- Linearity: 20 – 400 mg/L at 37°C as D-Lactic acid
- Sample/reagents: 1/40/1

Let reagents reach the working temperature before using.

Pipette in a test tube or cuvette so labeled:

R/B: Reagent Blank, S: Sample:

	R/B	S
*Working reagent	1000 µl	1000 µl
Distilled water	25 µl	---
Sample	---	25 µl

Mix and incubate for about 3 minutes at 37°C. Measure the absorbance AS1 and AR/B1. Then add:

*Reagent 3	25 µl	25 µl
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Mix carefully, incubate at 37°C and wait the end of the reaction (10-25 min.). Read AS2 and AR/B2.

Calculate for the sample AS = (AS2 - AS1); calculate for the Reagent/Blank AR/B = (AR/B2 - AR/B1).

Calculate the difference $\Delta A = AS - AR/B$.

CALCULATION

Use this general formula to calculate the concentration:

D-Lactic concentration (g/L) = $V/v \times 1/\epsilon d \times MW/1000 \times \Delta A$

V = total test volume = 1.050 mL

v = sample volume = 0.025 mL

d = pathlength = 1 cm

ϵ = molar coeff. NADH = 6.3 L / mmol x cm

MW = lactic acid MW = 90.1

so it becomes:

D-Lactic acid conc. (g/L) = 0.601 x ΔA

NOTE

1. The *Reagent 1 is supplied in surplus.
2. A proportional variation of the reaction volumes does not change the results.
3. For concentrations higher than the limit of Linearity of the different applications, dilute the sample with distilled water in the mentioned ranges; repeat the determination and multiply the result by the dilution factor.
4. We suggest do not mix Reagents from different Production lots.
5. **PAY ATTENTION!** Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. For fat containing samples please ask for specific procedure.
7. For solid or semi-solid samples please ask for specific procedure, eventual Carrez solutions pretreatment and Calculation.
8. Specificity: this test is specific for D-Lactic Acid. No interference was seen.

Ver.2007/10