Enzymatic method for wines, food and beverages
2 x 60 mL R1 + 5 x 20 mL R2 + 1 x 2.5 mL R3 + 1 x 10 mL R4
(50 tests on manual photometer, 500 tests on automatic analyzer)

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

Principle

Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate with simultaneous oxidation of NADH to NAD. The decrease in absorbance of NADH is directly proportional to the concentration of pyruvate in the sample.
Using the standard contained in the kit, you can prepare a calibration curve to refer to. By reporting the absorbance values and concentrations of the individual points on it, the concentrations of the individual samples can be determined.

Assay specifications

Wavelength: 340 nm (334 - 365 nm)
Path width: 1.00 cm (glass; plastic)
Temperature: 37 °C
Method: Endpoint
Reaction time: 3+5 (10) minutes
Measurement: Against air or distilled water
Linearity: 10 - 400 mg/L

Reagents

#1: R1 - Buffer (> 400 mmol/L): 2 vials of approx. 60 mL

#2: R2 - NADH (LDH > 250 KU/L): 5 vials of approx. 20 mL

#3: R3 - LDH Ready to use (NADH > 0.1 mmol/L): 1 vial of approx. 2.5 mL

#4: R4 - Liquid Standard: 1 vial of approx. 10 mL (pyruvate solution = 400 mg/L)

All reagents are ready to use. Bring the reagents to working temperature before use. Stir gently before adding. Close immediately after use.

This product has been formulated for in vitro diagnostic use. The reagent should only be used for the purpose indicated by experienced and trained personnel. The reagents contain sodium azide as a preservative, in a total concentration below the limits set out in Dir.67/548/EEC and 88/379/EEC and related amendments for the classification, labelling and packaging of dangerous preparations (reagents).

Do not ingest. Avoid contact with skin and mucous membranes. On the material safety data sheet are detailed the operating procedures for the manipulation of this product. Material safety data sheet can be supplied on request.

After use, the reagents must be disposed of as laboratory waste.

Stability:

Closed reagents are stable until the expiration date indicated on the label, when stored in their undamaged primary container between 2 and 8 °C, provided that they have not been contaminated during their use. If the primary container is damaged, dispose of it.

Preparation of the working reagent:

Dissolve one vial of **R2 - NADH** with 20 mL of **R1 - BUFFER,** stirring gently to avoid foaming. Close immediately after use. The products must be handled in such a way as to avoid any contamination. Close immediately after use.

Stability of the working reagent

The WORKING REAGENT is stable:

- 7 days at 2 - 8 °C;
- 60 days at -20 °C. Freeze once.

Sample preparation

- Wine can be analyzed directly.
- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range 10 - 400 mg/L.
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide.
- Crush and homogenize solid samples, weigh out appropriate sample amount and extract with water.

Test Procedure

Pipette into cuvettes:	Reagent Blank	Sample	Standard
Working reagent (1+2)	2000 µL	2000 µL	2000 µL
Water	50 µL	-	
R4 - CAL	-	-	50 µL
Sample	-	50 µL	
Mix and incubate for about 3 minutes at 37 °C. Measure the absorbance A1 of Reagent Blank, Sample and Standard. Then add:			
Reagent 3 - LDH	50 µL	50 µL	50 µL
Mix gently. Measure the absorbance A2 of Reagent Blank, Sample and Standard after 5 - 10 minutes at 37 °C.			

Calculation of results

Use this general formula to calculate the concentration:

$$\Delta A_{Sample} = (A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{REAGENT BLANK}}$$

$$\Delta A_{Standard} = (A_2 - A_1)_{\text{Standard}} - (A_2 - A_1)_{\text{REAGENT BLANK}}$$

Use this general formula to calculate concentration:

$$\text{Pyruvic acid (mg/L)} = \frac{V \times MW}{\epsilon \times d \times v} \times \Delta Abs$$

V = total test volume = 2.100 mL
v = sample volume = 0.050 mL
d = Optical path = 1 cm
ε = coeff. molar NADH = 6.3 L / mmol x cm
MW = 88.1 g/mol

So it becomes: **Pyruvic acid (mg/L) = 587 x Δ Abs**

Performance data

1. This test is specific to pyruvic acid. No interference is known.
2. Linearity of method :
   The test is linear between 10 and 400 mg/L. For pyruvate concentrations greater than 400 mg/L, dilute the sample with distilled water to bring the concentration into the mentioned range, repeat the determination, and multiply the result by the dilution factor. For concentrations below 10 mg/L, the sample can be increased from 0.050 to 0.200 mL, while R_{1+2} remains unchanged (2000 µL) and V changes to 2.250 mL. In this situation (sample volume = 0.200 mL), calculation will become:
   Pyruvic acid (mg/L) = 157.3 x Δ Abs
   In this case, the lower limit drops to 2 mg/L.
3. Applications on automatic chemistry analyzers are available upon request.

References

1. Methods of Enzymatic Analysis, Ed. by H.U.Bergmeyer, 3nd ed., Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel (1985).
2. Marbach E.P., Clin. Chem. 13, 314 (1967).

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