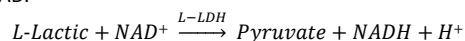


PURPOSE OF THE TEST

L-Lactic acid appears as a product of malolactic fermentation by the action of lactic acid bacteria in secondary fermentation. This process reduces perceived acidity as L-Malic is transformed into the softer L-Lactic, adding dairy flavour (milk, butter, cheese) which is desirable in many red wines, as it provides a feeling of mouth fullness.


METHOD

L-Lactate dehydrogenase (L-LDH) catalyzes the conversion of L-lactic to pyruvic with reduction of NAD.



The increase in absorbance at 340 nm due to the formation of NADH is proportional to the concentration of lactic acid.

CONTENT

R1	2 x 30 mL	 TRIS 200 mM, pH 9.0, L-LDH (>50 U/mL) ATENCIÓN: <i>H317. Sensibilización cutánea, categoría 1. Puede provocar una reacción alérgica en la piel. P302+P352: EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes. P333+P313: En caso de irritación o erupción cutánea: Consultar a un médico.</i>
R2	1 x 15 mL	NAD ⁺ 20 mM preservatives
CTRL	1 x 3 mL	L-Lactic Acid control 1,5 g/L (1,27 – 1,73 g/L)

REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 2-8 °C. Do not freeze.

Discard if absorbance of blank is higher than 0.500 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO₂ introduces instability in the measure. Samples containing CO₂ must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpyrrolidone (PVPP 0.1g for each 10 mL) to reduce the level of colour. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

PROCEDURE OVERVIEW

Treat standard, controls and samples as Sample. Use distilled water as Blank.

Use WINECONTROL (code SD2200) or WINECALRTU (code SY2100R) as standard.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Test Reaction
Reagent 1	720 µL	720 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37°C for 1 minutes and read absorbance at 340 nm (A₁). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37°C and read absorbance at 340 nm (A₂).

Concentration of L-Lactic acid is calculated as:

$$L\text{-Lactic} = \frac{(A_2 - 0.80 \times A_1)_{\text{sample}} - (A_2 - 0.80 \times A_1)_{\text{blank}}}{(A_2 - 0.80 \times A_1)_{\text{standard}} - (A_2 - 0.80 \times A_1)_{\text{blank}}} \times C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for L-Lactic.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	150	240
Name	L-LACTIC	
Method	End Point A	
Direction	Increasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 240]	3 - 4	4 - 5
Reading cycle [150 240]	20 - 21	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0.04 ~ 3.00	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 3.00 g/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of quantification (LoQ): 0.04 g/L

Limit of linearity: 3.00 g/L

NOTES

Using a control sample on a regular basis provides information on the calibration status and possible deterioration of the reagent. In case of deviations greater than 15% on the target value, it is advisable to check the calibration status of the test.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008). Official OIV method OIV-MA-AS313-07
2. Bermejer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY. (1974).
3. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Wine analysis and production. Van Nostrand Reinhold, 1st Ed. (1990).
4. Resolution OIV-OENO 598-2018. Determination of L-Lactic in wines by automated enzymatic method. (2018)

