SD6023: D-LACTIC ACID

ENZYMATIC – D-LDH

FOR USE IN WINE SAMPLES

PURPOSE OF THE TEST

Most of the lactic acid present in wine is produced mainly after malolactic fermentation due to transformation of L-Malic into L-Lactic, thus up to over 75% of the lactic present in wine is L isomer. D-Lactic, in the other hand is associated to the glucose (and other hexoses) metabolism by these same lactic bacteria (mostly *Leuconostoc* and *Lactobacillus*). Presence of D-Lactic higher than 0,3 g/L is a sign of bacterial contamination. As these lactic bacteria compete with yeast for sugars, it could even inhibit alcoholic fermentation.

METHOD

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

$$D\text{-}Lactic + NAD^+ \xrightarrow{D-LDH} Pyruvate + NADH + H^+$$

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, D-LDH (>50 U/mL)
R2	1 x 15 mL 	NAD^+ 20 mM, preservatives *WARNING: H302: Harmful if swallowed. P301+P310: IF SWALLOWED: Immediately call a POISON CENTER/doctor.*
STD	1 x 3 mL	D-Lactic acid 0,3 g/L (0,25 – 0,34 g/L)

REAGENT PREPARATION

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

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

SAMPLES

For use with wine samples.

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (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.

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 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm (A_2).

Concentration of D-Lactic is calculated as:

$$D\text{-}Lactic = \frac{(A_2 - 0.80xA_1)_{\text{sample}} - (A_2 - 0.80xA_1)_{\text{blank}}}{(A_2 - 0.80xA_1)_{\text{standard}} - (A_2 - 0.80xA_1)_{\text{blank}}} \; x\; C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the label for tartaric acid.

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

Dionysos model	**150**	**240**
Name	D-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	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0.02 ~ 0.60	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	—	
Vol. Sample Dil. Auto	—	

Procedure is linear up to 0.6 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): 0.02 g/L
Limit of linearity: 0.6 g/L

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008). Method MA-AS313-12A
2. Bermeyer, 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).