

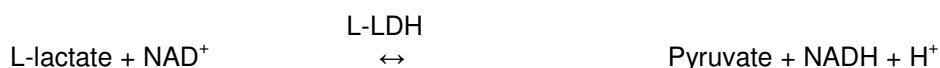
## ENZYMATIC TEST KIT FOR THE DETERMINATION OF L-LACTIC ACID IN GRAPE JUICE AND WINE

### PRODUCT

Product no. 4A150, for **30 tests**, for *in vitro* use only.

### PRINCIPLE OF MEASUREMENT

L-lactic acid is produced by lactic acid bacteria. Levels of L-lactic acid in wines may be monitored during malo-lactic fermentation, or to detect undesirable contamination by this bacterium. L-lactic acid content is determined enzymatically according to the following equations:



L-lactic acid is oxidised by nicotinamide adenine dinucleotide (NAD) to pyruvate using L-lactate dehydrogenase (L-LDH) enzyme as a catalyst. The equilibrium does not favour formation of pyruvate and so pyruvate is removed by a trapping enzyme. The amount of NADH formed is measured at 340 nm and is stoichiometrically related to the amount of L-lactate consumed. In this method, glutamate pyruvate transaminase (GPT) is used as the trapping enzyme.



### CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	33 mL	2 years at 4°C
2	NAD	Add 6.6 mL of distilled water, mix to dissolve	6.6 mL	2 years at 4°C (diluted: 1 year at 4°C, 2 years at -20°C)
3	GPT	Mix gently by swirling before use	0.7 mL	1 year at 4°C
4	L-LDH	Nil	0.7 mL	1 year at 4°C
5	Standard	Nil	3.3 mL	2 years at 4°C

The shelf life of Reagents 1 & 2 can be extended by placing aliquots in a freezer.

Do not freeze enzyme reagents 3 & 4.

Failure to store reagents at the recommended temperature will reduce their shelf life.

For concentration of Standard, refer to label on bottle.

### SAFETY

- **Wear safety glasses**
- **Reagent 1 is mildly corrosive**
- **Do not ingest Buffer or Standard as they contain sodium azide as a stabilizer**

### PROCEDURE

Operating Parameters

Wavelength	340 nm
Cuvettes	1cm, quartz, silica, methacrylate or polystyrene
Temperature	20 – 25°C
Final volume in cuvette	2.24 mL
Zero	against air without cuvette in light path

## SAMPLE PREPARATION

Samples should be diluted with water to ensure concentration in the assay solution is no more than 0.2 g/L. For samples with less than 2 g/L of L-lactic acid, a 1 in 10 dilution is appropriate. As a general guide, further dilution is required if the  $A_2$  absorbance reading is greater than 1.10 absorbance units.

Undiluted red wines or highly coloured undiluted juice samples may require decolourisation. To decolourise, add approximately 0.1 g of PVPP to 5 mL of sample in a test tube. Shake well for about 1 minute. Clarification is achieved by settling or filtering through Whatman No. 1 filter paper.

## SAMPLE ANALYSIS

a. Pipette the following volumes of reagents into the cuvettes:

Reagent	Blank	Standard	Sample
1. Buffer	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)
2. NAD	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)
Distilled water	1.00 mL (1000 $\mu$ L)	0.90 mL (900 $\mu$ L)	0.90 mL (900 $\mu$ L)
3. GPT	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)
Sample/Standard		0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)

b. Mix well by gentle inversion and read absorbances,  $A_1$ , after 3 minutes.

c. Pipette the following reagent into the cuvettes:

4. L-LDH	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)
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d. Mix well by gentle inversion and read absorbances,  $A_2$ , after 20 minutes.

## CALCULATIONS\*

1. Calculate the Net Absorbance for the Blank, Sample and Standard:

$$\text{Net Absorbance, } A_N = A_2 - A_1$$

2. Calculate the Corrected Absorbance by subtracting the Net Absorbance for the Blank from the Net Absorbance for the Sample:

$$\text{Sample Corrected Absorbance, } A_C = \text{Sample } A_N - \text{Blank } A_N$$

3. Do the same for the Standard by substituting the Standard absorbances in place of the Sample absorbances.

4. Calculate the L-Lactic acid concentration as follows:

$$\text{Lactic Acid Concentration (g/L)} = A_C \times 0.3203 \times \text{Dilution Factor}$$

\*A calculation spreadsheet is available for download at:

<http://www.vintessential.com.au/certification/calculation-worksheets/>

## REFERENCES

1. Bergmeyer, H.U. *et al* 1984, *Methods of Enzymatic Analysis*, 3<sup>rd</sup> ed., vol. 6, pp. 582-588; Verlag Chemie, Weinheim.

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