32 Brasser Avenue
Dromana Victoria 3936
Australia
T +61 3 5987 2242
F +61 3 5987 3303
E info@vintessential.com.au
W www.vintessential.com.au

ABN: 60 068 057 045

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:

$$\text{L-lactate} + NAD^+ \xleftrightarrow{\text{L-LDH}} \text{Pyruvate} + NADH + H^+$$

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.

$$\text{Pyruvate} + \text{L-glutamate} \xleftrightarrow{\text{GPT}} \text{L-alanine} + \text{2-oxoglutarate}$$

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 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
2. NAD	0.20 mL (200 µL)	0.20 mL (200 µL)	0.20 mL (200 µL)
Distilled water	1.00 mL (1000 µL)	0.90 mL (900 µL)	0.90 mL (900 µL)
3. GPT	0.02 mL (20 µL)	0.02 mL (20 µL)	0.02 mL (20 µL)
Sample/Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

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 (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

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*, 3rd ed., vol. 6, pp. 582-588; Verlag Chemie, Weinheim.