

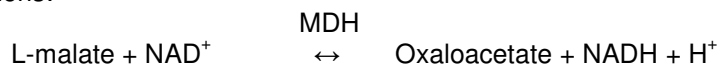
## ENZYMATIC TEST KIT FOR THE DETERMINATION OF L- MALIC ACID IN GRAPE JUICE AND WINE

### PRODUCT

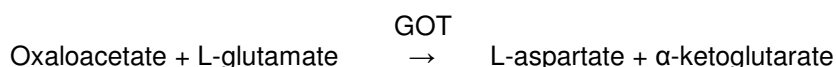
Product no. 4A165, for **100 tests**, for *in vitro* use only.

### PRINCIPLE OF MEASUREMENT

L-malic acid is found in grape juice and wine and is determined enzymatically according to the following equations:



L-malic acid is oxidised by nicotinamide adenine dinucleotide (NAD) to oxaloacetate using L-malate dehydrogenase (MDH) enzyme as a catalyst. The equilibrium does not favour formation of oxaloacetate and so oxaloacetate 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-malate consumed. In this method, glutamate oxaloacetate transaminase (GOT) is used as the trapping enzyme. In the presence of L-glutamate, the oxaloacetate is irreversibly converted to L-aspartate.



### CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	2 x 53 mL	2 years at 4°C
2	NAD	Add 22.0 mL of distilled water, mix to dissolve	22.0 mL	2 years at 4°C (diluted: 1 year at 4°C, 2 years at -20 °C)
3	GOT	Swirl gently before use	1.3 mL	2 years at 4°C
4	MDH	Swirl gently before use	1.3 mL	2 years 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	2.22 mL
Zero	against air without cuvette in light path

## SAMPLE PREPARATION

Samples should be diluted with distilled water to ensure that the concentration in the assay solution is no more than 0.4 g/L. For samples with less than 2 g/L of L-Malic acid, a 1 in 5 dilution is sufficient. As a general guide, further dilution is required if the absorbance reading at  $A_2$  is greater than 1 absorbance unit.

Undiluted red wines or highly coloured undiluted juice samples will 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, centrifugation, or by filtering through Whatman No. 1 filter paper.

## SAMPLE ANALYSIS

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

Reagent	Blank assay	Standard assay	Samples
1. Buffer	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)
Distilled water	1.00 mL (1000 $\mu$ L)	0.90 mL (900 $\mu$ L)	0.90 mL (900 $\mu$ L)
2. NAD	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)
3. GOT	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)
Sample or 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. MDH	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)
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d. Mix well by gentle inversion and read absorbances,  $A_2$ , after 10 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-Malic acid concentration as follows;

$$\text{Malic Acid Concentration (g/L)} = A_C \times 0.4725 \times \text{Dilution Factor}$$

5. Precision (where x is the malic acid concentration in the sample in g/l):

$$\text{Repeatability } r = 0.03 + 0.034x \quad \text{Reproducibility } R = 0.05 + 0.071x$$

\*A calculation spreadsheet is available for download at:

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

## REFERENCES

1. "Compendium of International Methods of Wine and Must Analysis" OIV, Vol 1, 2006, MA-E-AS313-11-ALMENZ, p3.

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