

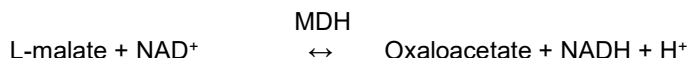
## **ENZYMATIC TEST KIT FOR THE DETERMINATION OF L-MALIC ACID IN GRAPE JUICE AND WINE**

### **PRODUCT**

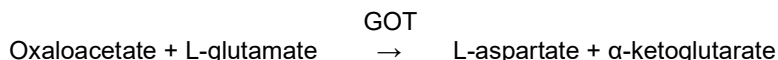
Product no. 4A165, for 100 tests, for *in vitro* use only.

### **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	All reagents (as provided) are stable for 24 months at 4°C or until the kit's expiry date, whichever occurs first. Reagent 2 (NAD) is stable for 1 year at 4°C or 2 years at -20 °C <i>once dissolved</i> or until the kit's expiry date, whichever occurs first.
2	NAD	Add 22 mL of distilled water & mix by inversion to dissolve	22 mL	
3	GOT	Swirl gently before use	1.3 mL	
4	MDH	Swirl gently before use	1.3 mL	
5	Standard	Nil	3.3 mL	

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 temperatures will reduce their shelf life.

For concentration of Standard (reagent 5), please refer to the label on the 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 ( $A_N$ ) for the Blank, Standard, and sample assays:

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

2. Calculate the Corrected Absorbance ( $A_C$ ) for the Standard assay by subtracting the Net Absorbance for the Blank from the Net Absorbance for the Standard:

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

3. Calculate the Corrected Absorbance ( $A_C$ ) for the samples by subtracting the Net Absorbance for the Blank from the Net Absorbance for the sample:

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

4. Calculate the L-Malic acid concentration for the Standard and samples as follows:

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

5. Precision (where  $x$  is the L-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 the following locations.

Australia based users

<https://winechek.com/calculation-worksheets/>

Users outside of Australia

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

## REFERENCES

1. OIV, 2018, Compendium of international methods of wine and must analysis. *International Organisation of Vine and Wine*, Vol 1: Paris, France, pp. OIV-MA-AS313-11.