Manufactured by Winechek Pty Ltd
1/22 Hightech Place Lilydale, Vic 3140

www.winechek.com
support@winechek.com

VINTESSENTIAL® TEST KITS

Winechek
PRECISION BASED EXPERTISE

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:

$$\text{L-malate} + NAD^+ \xleftrightarrow{\text{MDH}} \text{Oxaloacetate} + NADH + H^+$$

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.

GOT
$$\text{Oxaloacetate} + \text{L-glutamate} \xrightarrow{\text{GOT}} \text{L-aspartate} + \alpha\text{-ketoglutarate}$$

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 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
Distilled water	1.00 mL (1000 µL)	0.90 mL (900 µL)	0.90 mL (900 µL)
2. NAD	0.20 mL (200 µL)	0.20 mL (200 µL)	0.20 mL (200 µL)
3. GOT	0.01 mL (10 µL)	0.01 mL (10 µL)	0.01 mL (10 µL)
Sample or 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. MDH	0.01 mL (10µL)	0.01 mL (10µL)	0.01 mL (10µL)

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:

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.

Sample Corrected Absorbance, A_C = Sample A_N – 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;

Malic Acid Concentration (g/L) = A_C x 0.4725 x Dilution Factor

5. Precision (where x is the malic acid concentration in the sample in g/l):
Repeatability r = 0.03 + 0.034x *Reproducibility* R = 0.05 + 0.071x

*A calculation spreadsheet is available for download at:
http://www.vintessential.com.au/certification/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.