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:

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

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.

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

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	2 x 53 mL	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 *once dissolved* 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 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 (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):

Repeatability $r = 0.03 + 0.034x$ 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.