

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

PRODUCT

Product no. **4A160**, for **60 tests**, for *in vitro* use only.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	33 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 6.6 mL of distilled water & mix by inversion to dissolve	6.6 mL	
3	GOT	Swirl gently before use	0.4 mL	
4	MDH	Swirl gently before use	0.4 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 <i>micro-cuvette</i> , quartz, silica, methacrylate or polystyrene Re-ordering code 2C890
Temperature	20 – 25°C
Final volume in cuvette	1.11 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 A₂ 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	500 µL	500 µL	500 µL
Distilled water	500 µL	450 µL	450 µL
2. NAD	100 µL	100 µL	100 µL
3. GOT	5 µL	5 µL	5 µL
Sample or Standard		50 µL	50 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	5µL	5µL	5µL
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d. Mix well by gentle inversion and read absorbances, A_2 , after 10 minutes.

CALCULATIONS*

These may be performed on the Absorbance one software directly, or using the calculation spreadsheets below*

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:

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

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

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 in the absence of Absorbance one software.

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.