VINTESSENTIAL® TEST KITS
Manufactured by Winechek Pty Ltd
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Winechek
PRECISION BASED EXPERTISE

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

# FOR DISCRETE ANALYSERS

## PRODUCT

Product no.4B160, 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:

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

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:

| | | |
|---|---|---|
| MA R1 | Buffer | 19.5mL x 2 |
| MA R2 | $NAD^+$ | 5.5mL x 2 |
| MA R3 | GOT/MDH | 5.5mL x 2 |

Reagents are stable refrigerated at 4ºC until the 'best before' date printed on the batch label of the kit.
DO NOT FREEZE. Failure to store reagents at the recommended temperature will reduce their shelf life.

If decanting reagents into instrument-specific bottles, then please regularly rinse the bottles with distilled water and dry before adding fresh reagents. Failure to do this may reduce reagent shelf life due to a build-up of waste product.

## SAFETY

- Please read the Safety Data Sheets (SDS) before use;
- Take the necessary precautions for the use of laboratory reagents;
- The reagents contain sodium azide as preservative. DO NOT swallow. Avoid contact with skin and mucous membranes.

## PROCEDURE

### Reagent Definition

| Reagent | MA R1 | MA R2 | MA R3 |
|---|---|---|---|
| Stable on board (days) | 1 | 1 | 1 |
| Alarm limit (mL) | 1.0mL | 0.7mL | 0.7mL |
| Vial volume | 20mL | 20mL | 20mL |
| Syringe speed | Normal | Normal | Normal |

### Test Definition

| | |
|---|---|
| Test type | Photometric |
| Full name | L-Malic acid |
| Result unit | g/l |
| Number of decimals | 2 |
| Acceptance | Manual |
| Dilution 1+ | 0 |
| Sample type | Wine, Must, Juice |

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## Calibration Parameters

*For best results daily calibration is recommended*

| | |
|---|---|
| Calibration type | Linear |
| Repeat time (d) | 1 |
| Points/Calibrator | Duplicate |
| Acceptance | Manual |
| Curve direction | Ascending |
| Type of calibrators | Separate |

## Test Flow

| Calibrator | Conc. (g/l) | Dil. Ratio 1+ |
|---|---|---|
| MA 0.00 | 0.000 | 0.0 |
| MA 0.10 | 0.100 | 0.0 |
| MA 0.50 | 0.500 | 0.0 |
| MA 1.00 | 1.000 | 0.0 |
| MA 1.50 | 1.500 | 0.0 |
| MA 3.00 | 3.000 | 0.0 |

| Reagent | Reagent | Incubation | Sample | Incubation | Blank | Reagent | Incubation | End point |
|---|---|---|---|---|---|---|---|---|
| Reagent | Reagent | Time (sec.) | Volume (µl) | Time (sec.) | Resp. min | Reagent | Time (sec.) | Wave. (nm) |
| MA R1 | MA R2 | 60 | 2 | 60 | * | MA R3 | 300 | 340 |
| Volume (µl) | Volume (µl) | | Disp. with | | Resp. max | Volume (µl) | | Side wave. |
| 75 | 20 | | Water | | * | 20 | | NONE |
| Disp. with | Disp. with | | Volume (µl) | | | Disp. with | | |
| Water | Water | | 50 | | | Water | | |
| Volume (µl) | Volume (µl) | | Wash reagent | | | Volume (µl) | | |
| 43 | 30 | | NONE | | | 10 | | |
| Wash reagent | Wash reagent | | | | | Wash reagent | | Meas. type |
| NONE | NONE | | | | | NONE | | NORMAL |

## 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.

**AUSTRALIAN-MADE**

This test kit was made with pride in a lab down-under.

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