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

FOR DISCRETE ANALYSERS

PRODUCT

Product no.4B160, **for 500 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.

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

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Quantity	Stability
REAGENT 1	Buffer	19.5mL x 2	24 months at 4°C
REAGENT 2	NAD	5.5mL x 2	12 months at 4°C
REAGENT 3	GOT/MDH	5.5mL x 2	6 months at 4°C

The shelf life of Reagents R1 & R2 can be extended by placing aliquots in a freezer.
DO NOT FREEZE Reagent R3 (GOT/MDH).
Failure to store reagents at the recommended temperature will reduce their shelf life.

SAFETY

- **Wear safety glasses**
- **Reagent R1 is mildly corrosive**
- **The reagents contain sodium azide as preservative. Do not ingest.**

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

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

Calibrator	Conc. (g/l)	Dil. Ratio 1+
Water	0.000	0.0
MA 0.10	0.100	0.0
MA 0.25	0.250	0.0
MA 0.50	0.500	0.0
MA 1.00	1.000	0.0
MA 3.00	3.000	0.0

Test Flow

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. “Compendium of International Methods of Wine and Must Analysis” OIV, Vol 1, 2006, MA-E-AS313-11-ALMENZ, p3.

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