32 Brasser Avenue
Dromana Victoria 3936
Australia
T +61 3 5987 2242
F +61 3 5987 3303
E info@vintessential.com.au
W www.vintessential.com.au

ABN: 60 068 057 045

ENZYMATIC TEST KIT FOR THE DETERMINATION OF ACETIC ACID IN GRAPE JUICE AND WINE

FOR DISCRETE ANALYSERS

PRODUCT
Product no. 4B100, for *in vitro* use only.

PRINCIPLE OF MEASUREMENT
Acetic acid can be a spoilage indicator in wine and is limited by regulation in most wine producing countries.
It can be determined enzymatically by monitoring the reaction that produces NADH, according to the following equations:

$$\text{Acetic acid} + \text{ATP} + \text{CoA} \xrightarrow{\text{ACS}} \text{acetyl-CoA} + \text{AMP}^2 + \text{pyrophosphate}$$

In the presence of coenzymes Adenosine-5'-triphosphate (ATP) and Coenzyme A (CoA), the acetic acid is converted to acetyl-CoA by the enzyme Acetyl-CoA-synthetase (ACS). Catalysed by the enzyme Citrate synthase (CS), the acetyl-CoA then reacts with oxaloacetate to product citrate and CoA:

$$\text{Acetyl-CoA} + \text{oxaloacetate} + H_2O \xrightarrow{\text{CS}} \text{citrate} + \text{CoA}$$

The oxaloacetate required for the reaction is formed from malate and nicotinamide-adenine dinucleotide (NAD) in the presence of malate dehydrogenase (MDH). In this reaction, NAD is reduced to NADH:

$$\text{Malate} + NAD^+ \xrightarrow{\text{MDH}} \text{oxaloacetate} + \text{NADH} + H^+$$

The amount of NADH formed is measured at 340 nm. Because the preceding indicator reaction catalysed by MDH is an equilibrium reaction, the amount of NADH formed is not linearly proportional to the acetic acid concentration in the assay. Therefore the calibration curve employed is nonlinear or point-to-point.

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Quantity	Stability
REAGENT 1	Buffer	17.5mL x 2	18 months at 4°C
REAGENT 2	(ATP/CoA/NAD)	5.5mL x 2	12 months at 4°C
REAGENT 3	CS/MDH	5.5mL x 2	9 months at 4°C
REAGENT 4	ACS	5.5mL x 2	9 months 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.

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

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PROCEDURE

Reagent Definition

Reagent	AA R1	AA R2	AA R3	AA R4
Stable on board (days)	1	1	1	1
Alarm limit (mL)	1.0mL	0.7mL	0.7mL	0.7mL
Vial volume	20mL	20mL	20mL	20mL
Syringe speed	Normal	Normal	Normal	Normal

Test Definition

Test type	Photometric
Full name	Acetic Acid
Result unit	g/l
Number of decimals	2
Acceptance	Manual
Dilution 1+	9.0
Sample type	Wine, Must, Juice

Calibration Parameters

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

Calibrator	Conc. (g/l)	Dil. Ratio 1+
AA 0.00	0.00	9.0
AA 0.10	0.10	9.0
AA 0.50	0.50	9.0
AA 1.00	1.00	9.0
AA 1.50	1.50	9.0
AA 2.00	2.00	9.0

Test Flow

Reagent	Reagent	Sample	Incubate	Reagent	Incubate	Blank	Reagent	Incubate	End point
Reagent	Reagent	Volume (µl)	Time (sec.)	Reagent	Time (sec.)	Resp.min	Reagent	Time (sec.)	Wave (nm)
AA R1	AA R2	4	60	AA R3	180	*	AA R4	300	340
Volume (µl)	Volume (µl)	Disp. with		Volume (µl)		Resp. max	Vol. (µl)		Side wave.
67	20	Water		20		*	20		NONE
Disp. with	Disp. with	Volume (µl)		Disp. with			Disp. with		
Water	Water	30		Water			Water		
Volume (µl)	Volume (µl)	Wash reagent		Volume (µl)			Vol. (µl)		
45	20	NONE		19			5		
Wash reagent	Wash reagent			Wash reagent			Wash		Meas. type
NONE	NONE			NONE			NONE		NORMAL