



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

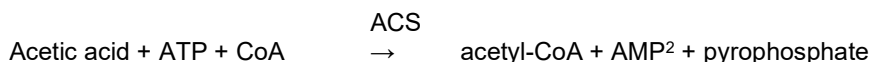
FOR DISCRETE ANALYSERS

PRODUCT

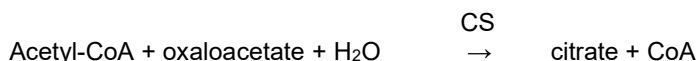
Product no. 4B100, for *in vitro* use only.

PRINCIPLE OF MEASUREMENT

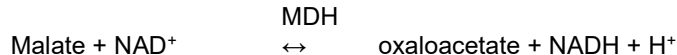
Acetic acid can be a spoilage indicator in wine and is limited by regulation in most wine producing countries. It can be determined enzymatically by monitoring the reaction that produces NADH, according to the following equations:



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:



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:



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:

AA R1	Buffer	17.5mL x 2
AA R2	Coenzymes (ATP/CoA/NAD ⁺)	5.5mL x 2
AA R3	CS/MDH	5.5mL x 2
AA R4	ACS	5.5mL x 2

Reagents are stable refrigerated at 4°C until the 'best before' date printed on the batch label.
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	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

Calibration Parameters

For best results daily calibration is recommended

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

Sample type	Wine, Must, Juice
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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
AA R1	AA R2	4	60	AA R3	180	*	AA R4	300	340
Volume (µl)	Volume (µl)	Disp. with		Volume (µl)		Resp. min	Volume (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

AUSTRALIAN-MADE

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