



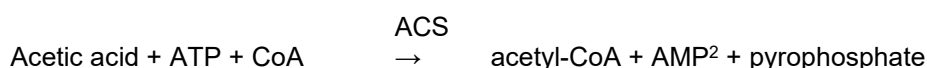
## **ENZYMATIC TEST KIT FOR THE DETERMINATION OF ACETIC ACID IN GRAPE JUICE AND WINE FOR DISCRETE ANALYSERS**

### **PRODUCT**

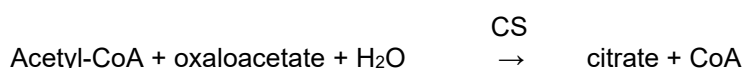
Product no. 4B100, for *in vitro* use only.

### **PRINCIPLE OF MEASUREMENT**

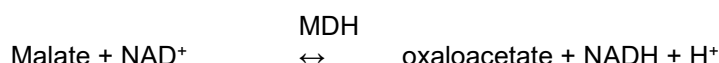
Acetic acid can be a spoilage indicator in wine and is limited by regulation in most wine producing countries. It can be determined enzymatically by monitoring the reaction that produces NADH, according to the following equations:



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 <sup>+</sup> ) | 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

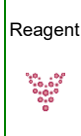
For best results daily calibration is recommended

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

|             |                   |
|-------------|-------------------|
| Sample type | Wine, Must, Juice |
|-------------|-------------------|

| 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                                                                             | 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                                                                                |

## AUSTRALIAN-MADE

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