

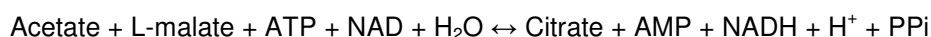
## **ENZYMATIC ANALYSIS KIT FOR THE DETERMINATION OF ACETIC ACID IN GRAPE JUICE AND WINE**

### **PRODUCT**

Product no. 4A105, for 100 tests, 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. In wine, it can be determined enzymatically by monitoring the reaction that produces NADH, according to the following overall equation:



The amount of NADH formed is measured at 340 nm and is stoichiometrically related to the amount of acetate consumed.

### **CONTENTS**

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	2 x 53 mL	18 months at 4°C
2	Coenzymes (ATP/CoA/NAD)	Nil	22 mL	18 months at 4°C
3	CS/MDH	Swirl gently before use	1.1 mL	18 months at 4°C
4	ACS	Swirl gently before use	2.2 mL	18 months at 4°C
5	Standard	Nil	3.3 mL	18 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.

For concentration of Standard, refer to label on bottle.

### **SAFETY**

- **Wear safety glasses**
- **Reagent 1 is mildly corrosive**
- **Do not ingest Buffer or Standard as they contain sodium azide as a stabilizer**

### **PROCEDURE**

Operating Parameters

Wavelength	340 nm
Cuvettes	1cm, quartz, silica, methacrylate or polystyrene
Temperature	20 – 25°C
Final volume in cuvette	3.23 mL
Zero	against air without cuvette in light path

## SAMPLE PREPARATION

Samples should be diluted with distilled water to ensure concentration in the assay solution is no more than 0.25 g/L. For most samples, a 1 in 5 dilution should be sufficient.

Ideally,  $A_3$  absorbance readings should be no greater than 1.20 absorbance units.

Undiluted red wines or highly coloured undiluted juice samples require decolourisation.

To decolourise, add approximately 0.1 g of PVPP to 5 mL of sample in a test tube. Shake well for about 1 minute. Clarification is achieved by settling, or filtering through Whatman No. 1 filter paper.

## SAMPLE ANALYSIS

a. Pipette the following volumes of reagents into the cuvettes:

Reagent	Blank	Standard	Sample
1. Buffer	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)
2. Coenzymes	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)	0.20 mL (200 $\mu$ L)
Distilled water	2.00 mL (2000 $\mu$ L)	1.90 mL (1900 $\mu$ L)	1.90 mL (1900 $\mu$ L)
Sample/Standard		0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)

b. Mix well by inversion and read absorbances,  $A_1$ .

c. Pipette the following reagent into the cuvettes:

3. CS/MDH	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)	0.01 mL (10 $\mu$ L)
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d. Mix well by inversion and read absorbances,  $A_2$ , after 3 minutes.

4. ACS	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)
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e. Mix well by inversion and read absorbances,  $A_3$ , after 5-10 minutes, or once the reaction is completed.

## CALCULATIONS\*

1. Calculate the absorbance differences for the Blank, Sample and Standard to give  $\Delta A_1$  and  $\Delta A_2$ :

$$\begin{aligned}\text{Absorbance difference, } \Delta A_1 &= A_2 - A_1 \\ \text{Absorbance difference, } \Delta A_2 &= A_3 - A_1\end{aligned}$$

2. Calculate the acetic acid content absorbance for the sample,  $\Delta A_{ac}$ , using the formula:

$$\Delta A_{ac} = [\Delta A_{2\text{sample}} - (\Delta A_{1\text{sample}})^2 \div \Delta A_{2\text{sample}}] - [\Delta A_{2\text{blank}} - (\Delta A_{1\text{blank}})^2 \div \Delta A_{2\text{blank}}]$$

3. Do the same for the Standard by substituting the Standard absorbances in place of the Sample absorbances.

4. Calculate the Acetic acid concentration as follows:

$$\text{Acetic Acid Concentration (g/L)} = \Delta A_{ac} \times 0.308 \times \text{Dilution Factor}$$

\*A calculation spreadsheet is available for download at:

<http://www.vintessential.com.au/certification/calculation-worksheets/>

## REFERENCES

1. Bergmeyer, H.U. *et al* 1984, *Methods of Enzymatic Analysis*, 3<sup>rd</sup> ed., vol. 6, pp. 639-645; Verlag Chemie, Weinheim.