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

PRODUCT
Product no. 4A100**, for 60 tests,** for *in vitro* use only.

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	**Preparation**	Quantity	Stability
1	Buffer	Nil	33 mL	Reagents are stable for 18 months at 4°C or until the kit's expiry date, whichever occurs first
2	Coenzymes (ATP/CoA/NAD)	Nil	6.6 mL	
3	CS/MDH	Swirl gently before use	0.4 mL	
4	ACS	Swirl gently before use	0.7 mL	
5	Standard	Nil	3.3 mL	

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 the concentration of the Standard, please refer to the bottle label.

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 *micro-cuvette*, quartz, silica, methacrylate or polystyrene Re-ordering code 2C890
Temperature	20 – 25°C
Final volume in cuvette	1.615 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 assay	Standard assay	Sample assays
1. Buffer	500 µL	500 µL	500 µL
Distilled water	1000 µL	950 µL	950 µL
2. Coenzymes	100 µL	100 µL	100 µL
Sample or Standard		50 µL	50 µL

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

c. Pipette the following reagent into the cuvettes:

3. CS/MDH	5µL	5µL	5µL

d. Mix well by inversion and read absorbances, A_2, after 3 minutes.

4. ACS	10µL	10µL	10µL

e. Mix well by inversion and read absorbances, A_3, after 20 minutes.

CALCULATIONS*

These may be performed on the Absorbance one software directly, or using the calculation spreadsheets below*

1. Calculate the absorbance differences ($A_2 - A_1$) and ($A_3 - A_1$) for the Blank, Standard and Samples to give ΔA_1 and ΔA_2:

Absorbance difference, ΔA_1 = $A_2 - A_1$
Absorbance difference, ΔA_2 = $A_3 - A_1$

2. Calculate the corrected absorbance for the samples and/or standard, $\Delta A_{ac,}$ using the formula:

$$\Delta A_{ac} = [(\Delta A_2)_{sample} - \frac{(\Delta A_1)^2_{sample}}{(\Delta A_2)_{sample}}] - [(\Delta A_2)_{blank} - \frac{(\Delta A_1)^2_{blank}}{(\Delta A_2)_{blank}}]$$

3. Calculate the Acetic acid concentration as follows:

Acetic Acid Concentration (g/L) = ΔA_{ac} x 0.308 x Dilution Factor

*A calculation spreadsheet is available for download at the following locations in the absence of Absorbance one software.

Australia based users
https://winechek.com/calculation-worksheets/

Users outside of Australia
http://www.vintessential.com.au/resources/calculation-worksheets/

REFERENCES

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