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ENZYMATIC TEST 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. 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:

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

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 acetic acid concentration must be calculated according to the equations on page 2 of these instructions.

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 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, 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 assay	Standard assay	Sample assays
1. Buffer	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
2. Coenzymes	0.20 mL (200 µL)	0.20 mL (200 µL)	0.20 mL (200 µL)
Sample or Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

b. Mix well by inversion and read absorbances, A_1.
c. Pipette the following reagent into the cuvettes:

3. CS/MDH	0.01 mL (10µL)	0.01 mL (10µL)	0.01 mL (10µL)

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

4. ACS	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

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

CALCULATIONS*

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, ΔA_{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:
http://www.vintessential.com.au/certification/calculation-worksheets/

REFERENCES

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