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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:

$$\text{Acetate} + \text{L-malate} + \text{ATP} + \text{NAD} + H_2O \leftrightarrow \text{Citrate} + \text{AMP} + \text{NADH} + H^+ + \text{PPi}$$

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	1 year at 4°C
2	Coenzymes (ATP/CoA/NAD)	Add 22 mL of distilled water, mix to dissolve	22 mL	1 year at 4°C (2 months at 4°C diluted)
3	CS/MDH	Mix gently by inversion before use	1.1 mL	1 year at 4°C
4	ACS	Mix gently by inversion before use	2.2 mL	1 year at 4°C
5	Standard	Nil	3.3 mL	1 year 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 0.2 g/L. For samples with a concentration higher than this, an appropriate dilution should be performed. As a general guide, absorbance readings should be no greater than 1 absorbance unit.

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 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
2. Coenzymes	0.20 mL (200 µL)	0.20 mL (200 µL)	0.20 mL (200 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
Sample/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 10-15 minutes, or once the reaction is completed. Note that samples with high alcohol contents can slow this reaction; allow 20 minutes in these cases.

CALCULATIONS*

1. Calculate the absorbance differences for the Blank, Sample and Standard 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 acetic acid content absorbance for the sample, $\Delta A_{ac,}$ using the formula:

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

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:

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

*A calculation spreadsheet is available for download at www.vintessential.com/enzyme_kits.htm

REFERENCES

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