VINTESSENTIAL® LABORATORIES

32 Brasser Avenue
Dromana Victoria 3936
Australia
T +61 3 5987 2242
F +61 3 5987 3303
E info@vintessential.com.au
W www.vintessential.com.au

ABN: 60 068 057 045

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

## PRODUCT

Product no. 4A126, **for 30 tests, for *in vitro* use only**

## PRINCIPLE OF MEASUREMENT

Citric acid may be used at the final stages of winemaking to make minor adjustments to acid levels without affecting the bi-tartrate stability of the wine. It is determined enzymatically according to the following equations:

$$\text{Citrate} \xrightarrow{\text{CL}} \text{oxaloacetate} + \text{acetate}$$

In the presence of enzymes MDH and LDH, both the oxaloacetate and its decarboxylation product pyruvate, are reduced by NADH to malate and lactate respectively.

$$\text{Oxaloacetate} + \text{pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{MDH/LDH}} \text{malate} + \text{lactate} + NAD^+$$

The amount of NADH oxidised is measured at 340 nm and is stoichiometrically related to the amount of citrate present.

## CONTENTS

The kit includes the following reagents:

| Reagent No. | Reagent | Preparation | Quantity | Stability |
|---|---|---|---|---|
| 1 | Buffer | Nil | 33 mL | 1 year at 4°C |
| 2 | NADH | Add 1.7 mL of distilled water to either bottle as required, mix to dissolve | 2 x 1.7 mL | 1 year at 4°C (1 month at 4°C once diluted) |
| 3 | MDH/LDH | Mix gently by inversion before use | 0.7 mL | 1 year at 4°C |
| 4 | CL | Add 0.35mL of distilled water to either bottle as required, mix to dissolve | 2 x 0.35mL | 1 year at 4°C (2 months at 4°C once diluted) |
| 5 | Standard | Nil | 3.3 mL | 1 year at 4°C |

The shelf life of Reagent 1 can be extended by placing aliquots in a freezer.
Do not freeze reagents 2, 3 or 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**
- **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 | 3.14 mL |
| Zero | against air without cuvette in light path |

## SAMPLE PREPARATION

Samples should be diluted to ensure concentration in the assay solution is no more than 0.5 g/L.
For most samples, a 1 in 2 dilution with distilled water should be sufficient.
For samples containing between 1 g/L to 2.5 g/L of citric acid, a 1 in 5 dilution would be appropriate.
Ideally, $A_1$ should lie between 0.90 – 1.20 absorbance units.

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. NADH | 0.10 mL (100 µL) | 0.10 mL (100 µL) | 0.10 mL (100 µL) |
| Distilled water | 2.00 mL (2000 µL) | 1.80 mL (1800 µL) | 1.80 mL (1800 µL) |
| Sample/Standard | | 0.20 mL (200 µL) | 0.20 mL (200 µL) |
| 3. MDH/LDH | 0.02 mL (20 µL) | 0.02 mL (20 µL) | 0.02 mL (20 µL) |

b. Mix well by gentle inversion and read absorbances, $A_1$, after 5 minutes.

c. Pipette the following reagent into the cuvettes:

| | | | |
|---|---|---|---|
| 4. CL | 0.02 mL (20µL) | 0.02 mL (20µL) | 0.02 mL (20µL) |

d. Mix well by gentle inversion and read absorbances, $A_2$, after 5 minutes.

## CALCULATIONS*

1. Calculate the Net Absorbance for the Blank, Sample and Standard:

$$\text{Net Absorbance, } A_N = A_1 - A_2$$

2. Calculate the Corrected Absorbance by subtracting the Net Absorbance for the Blank from the Net Absorbance for the Sample.

$$\text{Sample Corrected Absorbance, } A_C = \text{Sample } A_N - \text{Blank } A_N$$

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

4. Calculate the Citric acid concentration as follows;

$$\text{Citric acid (g/L)} = A_C \times 0.4787 \times \text{Dilution Factor}$$

*A calculation spreadsheet is available for download at:
http://www.vintessential.com.au/certification/calculation-worksheets/

## REFERENCES

1. Bergmeyer, H.U. *et al* 1985, *Methods of Enzymatic Analysis*, 3rd ed., vol. 7, pp. 2-12; Verlag Chemie, Weinheim.