ENZYMATIC TEST 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:

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

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.

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

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	Reagents are stable for 12 months at 4°C or until the kit's expiry date, whichever occurs first. Reagent 2 and 4 are stable for 1 month (Reagent 2) and 2 months (Reagent 4) at 4°C once dissolved or until the kit's expiry date, whichever occurs first.
2	NADH	Add 1.7 mL of distilled water to either bottle as required, mix to dissolve	2 x 1.7 mL	
3	MDH/LDH	Mix gently by inversion before use	0.7 mL	
4	CL	Add 0.35mL of distilled water to either bottle as required, mix to dissolve	2 x 0.35mL	
5	Standard	Nil	3.3 mL	

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 25 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 the following locations:

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

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

REFERENCES

1. OIV, 2018, Compendium of international methods of wine and must analysis. *International Organisation of Vine and Wine,* Vol 1: Paris, France, pp. OIV-MA-AS313-09.