

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

PRODUCT

Product no. 4A126, 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 | 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 | All reagents (as provided) are stable for 12 months at 4°C or until the kit's expiry date, whichever occurs first. Reagent 2 (NADH) is stable for 1 month at 4°C <i>once dissolved</i> and Reagent 4 (CL) is stable for 2 months at 4°C <i>once dissolved</i> or until the kit's expiry date, whichever occurs first. |
| 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 <i>micro-cuvette</i> , quartz, silica, methacrylate or polystyrene Re-ordering code 2C890 |
| Temperature | 20 – 25°C |
| Final volume in cuvette | 1.57 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 | 500 µL | 500 µL | 500 µL |
| 2. NADH | 50 µL | 50 µL | 50 µL |
| Distilled water | 1000 µL | 900 µL | 900 µL |
| Sample/Standard | | 100 µL | 100 µL |
| 3. MDH/LDH | 10 µL | 10 µL | 10 µL |

b. Mix well by gentle inversion, incubate for 5 minutes and read absorbances, A_1 .

c. Pipette the following reagent into the cuvettes:

| | | | |
|-------|------|------|------|
| 4. CL | 10µL | 10µL | 10µL |
|-------|------|------|------|

d. Mix well by gentle inversion, incubate for 25 minutes and read absorbances, A_2 ,

CALCULATIONS*

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

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 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. 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.