

## **ENZYMATIC TEST KIT FOR THE DETERMINATION OF D-GLUCOSE AND D-FRUCTOSE IN GRAPE JUICE AND WINE**

### **PRODUCT**

Product no. 4A145, for **100 tests**, for *in vitro* use only.

### **CONTENTS**

The kit includes the following reagents:

| Reagent No. | Reagent              | Preparation  | Quantity   | Stability  |
|-------------|----------------------|--|------------|--|
| 1           | Buffer               | To activate the Buffer, add the contents of Reagent No.2 Coenzymes (ATP/NADP) and mix with inversion until completely dissolved. | 2 x 53 mL  | All reagents (as provided) are stable for 18 months at 4°C or until the kit's expiry date, whichever occurs first. Reagent 1 (Buffer) is stable for 6 months at 4°C once activated or until the kit's expiry date, whichever occurs first. |
| 2           | Coenzymes (ATP/NADP) |  | 2 x 0.35 g |  |
| 3           | G6PDH/HK             | Swirl gently before use  | 2.2 mL     |  |
| 4           | PGI                  | Swirl gently before use  | 2.2 mL     |  |
| 5           | Standard             | Nil  | 3.3 mL     |  |

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 significantly reduce their shelf life. For concentration of the Standard, refer to the label on the 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 cuvette, quartz, silica, methacrylate or polystyrene |
| Temperature             | 20 – 25°C  |
| Final volume in cuvette | 3.04 mL  |
| Zero                    | against air without cuvette in light path                |

### **SAMPLE PREPARATION**

Samples should be diluted with distilled water to ensure that the concentration in the assay solution is no more than 1.0 g/L. For the majority of dry wine samples, a 1 in 10 dilution is satisfactory.

Semi-sweet wines may require up to a 1 in 50 dilution, while fortified and dessert wines may require up to a 1 in 100 dilution or greater. As a general guide, further dilution is required if the final A<sub>3</sub> absorbance reading is greater than 1.2 absorbance units. Samples may be used directly without decolourisation. Turbid samples must be either centrifuged or filtered through Whatman No. 1 filter paper to clarify.

## SAMPLE ANALYSIS

a. Check that Reagent No.1 Buffer has been activated by the addition of Reagent No.2 Coenzymes

a. Pipette the following volumes of reagents into the cuvettes:

| Reagent               | Blank assay | Standard assay | Sample assays |
|-----------------------|-------------|----------------|---------------|
| 1. Buffer             | 1000 µL     | 1000 µL        | 1000 µL       |
| 2. Distilled water    | 2000 µL     | 1900 µL        | 1900 µL       |
| 3. Sample or Standard |             | 100 µL         | 100 µL        |

b. Mix well by inversion and read absorbances,  $A_1$  after 3 minutes

c. Pipette the following reagent into the cuvettes:

|             |      |      |      |
|-------------|------|------|------|
| 3. G6PDH/HK | 20µL | 20µL | 20µL |
|-------------|------|------|------|

d. Mix well by inversion, incubate for 3 minutes and read absorbances,  $A_2$ ,

e. Pipette the following reagent into the cuvettes:

|        |      |      |      |
|--------|------|------|------|
| 4. PGI | 20µL | 20µL | 20µL |
|--------|------|------|------|

f. Mix well by inversion, Incubate for 10 minutes.and read absorbances,  $A_3$ ,

## CALCULATIONS\*

1. Calculate the Corrected Absorbance for the sample for D-Glucose:

$$\text{D-Glucose Absorbance, } A_G =$$

2. Calculate the D-Glucose concentration as follows:

$$\text{D-Glucose Concentration (g/L)} = \frac{(A_2 - A_1) - (\text{Blank}A_2 - \text{Blank}A_1)}{A_G \times 0.8637 \times \text{Dilution Factor}}$$

3. Calculate the Corrected Absorbance for the sample for D-Fructose:

$$\text{D-Fructose Absorbance, } A_F = (A_3 - A_2) - (\text{Blank}A_3 - \text{Blank}A_2)$$

4. Calculate the D-Fructose concentration as follows:

$$\text{D-Fructose Concentration (g/L)} = A_F \times 0.8694 \times \text{Dilution Factor}$$

5. Add the D-Glucose and D-Fructose results together to get the total residual sugar concentration

6. Do the same for the Standard by substituting the Standard absorbance values in place of the sample absorbance values.

7. Precision (where x is the D-glucose or D-fructose concentration in the sample in g/l):

$$\text{Repeatability } r = 0.056x \quad \text{Reproducibility } R = 0.12 + 0.076x$$

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