ENZYMATIC TEST KIT FOR THE DETERMINATION OF SUCROSE/GLUCOSE/FRUCTOSE IN GRAPE JUICE AND WINE

PRODUCT
Product no. 4A180**, for 30 tests,** for *in vitro* use only.

PRINCIPLE OF MEASUREMENT
Sucrose is hydrolysed by the enzyme β-fructosidase (invertase) to Glucose and Fructose:

$$\text{Sucrose} + H_2O \xrightarrow{\beta\text{-fructosidase}} \text{Glucose} + \text{Fructose}$$

Then, glucose and fructose are determined enzymatically according to the following equations:

$$\text{Glucose} + \text{ATP} \xrightarrow{HK} \text{Glucose-6-phosphate} + \text{ADP}$$
$$\text{Fructose} + \text{ATP} \rightarrow \text{Fructose-6-phosphate} + \text{ADP}$$

Glucose and fructose react with adenosine triphosphate (ATP) in the presence of the enzyme hexokinase (HK) to form glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P).

$$\text{G6P} + NADP^+ \xrightarrow{G6PDH} \text{Gluconate-6-phosphate} + NADPH + H^+$$

G6P is oxidised by nicotinamide adenine dinucleotide phosphate (NADP) to gluconate 6-phosphate using glucose-6-phosphate dehydrogenase (G6PDH) enzyme as a catalyst. The amount of NADPH formed is measured at 340 nm and is stoichiometrically related to the amount of glucose consumed.

$$\text{Fructose-6-phosphate} \xleftrightarrow{PGI} \text{Glucose-6-phosphate}$$

Next, the enzyme phosphoglucose isomerase (PGI) is added to convert the F6P to G6P. The G6P now formed reacts with NADP and the NADPH determined is stoichiometrically related to the amount of fructose present. The final result is therefore the combined concentration of sucrose, glucose and fructose in the sample. The sucrose content of the sample can be determined by subtracting the glucose & fructose content, as determined using Vintessential Laboratories Analysis Kit 4A140, from the sucrose/glucose/fructose content.

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1 2	Buffer Coenzymes (ATP/NADP)	To activate the Buffer, add the contents of Reagent No.2 Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	33 mL 0.2 g	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 and Reagent 5 is stable for 12 months or until the kit's expiry date, whichever occurs first.
3	G6PDH/HK	Swirl gently before use	0.7 mL	
4	PGI	Swirl gently before use	0.7 mL	
5	Invertase	Nil	0.7 mL	
6	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 & 5. 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

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	3.06 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 0.8 g/L. For the majority of dry wine samples, a 1 in 10 dilution is satisfactory. Semi-sweet wines, including sparkling wines may require a 1 in 20 or a 1 in 50 dilution dependant on the sucrose concentration. Fortified and dessert wines may require a 1 in 100 dilution or greater.
As a general guide, further dilution is required if the absorbance reading is greater than 1 absorbance unit. Samples may be used directly without decolourisation. Filter turbid samples through Whatman No. 1 filter paper.

SAMPLE ANALYSIS

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

Reagent	Blank assay	Standard assay	Sample assays
Distilled water	0.10 mL (100 µL)		
Sample or Standard		0.10 mL (100 µL)	0.10 mL (100 µL)
5. Invertase	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

b. Gently tap each cuvette to ensure the invertase is mixed with the sample. Incubate for 20 minutes. c. Pipette the following reagents into the cuvettes:

Reagent	Blank assay	Standard assay	Sample assays
Distilled water	1.90 mL (1900 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
2. Buffer/Coenzyme mix	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)

d. Mix well and read absorbances, A_1
e. Pipette the following reagent into the cuvettes:

3. G6PDH/HK	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

f. Mix well and read absorbances, A_2, after 10 minutes.
g. Pipette the following reagent into the cuvettes:

4. PGI	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

h. Mix well and read absorbances, A_3, after 10 minutes.

CALCULATIONS*

Equation 1 = $[(SampleA_2 – SampleA_1) – (BlankA_2 - BlankA_1)] \times 0.8693 \times$ Dilution Factor

Equation 2 = [(SampleA_3 – SampleA_2) – (BlankA_3 – BlankA_2)] x 0.8751 x Dilution Factor

Sucrose/Glucose/Fructose (g/L) = Equation 1 + Equation 2

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

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

To calculate the sucrose content, simply subtract the sugar concentration as determined by kit 4A140:

Sucrose Concentration (g/L) = (g/L of Sucrose/Glucose/Fructose) – (g/L of Glucose/Fructose)

www.vintessential.com.au

REFERENCES

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