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

PRODUCT
Product no. 4A140**, for 60 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	33 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)	Reagent No.2 Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	0.2 g	
3	G6PDH/HK	Swirl gently before use	0.7 mL	
4	PGI	Swirl gently before use	0.7 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 *micro-cuvette*, quartz, silica, methacrylate or polystyrene Re-ordering code 2C890
Temperature	20 – 25˚C
Final volume in cuvette	1.02 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_3 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	500 µL	500 µL	500 µL
2. Distilled water	1000 µL	950 µL	950 µL
3. Sample or Standard		50 µL	50 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	10µL	10µL	10µL

d. Mix well by inversion, incubate for 10 minutes and read absorbances, A_2,

e. Pipette the following reagent into the cuvettes:

4. PGI	10µL	10µL	10µL

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:

D-Glucose Absorbance, A_G =

2. Calculate the D-Glucose concentration as follows:

$(A_2 – A_1)$ – (BlankA_2 - BlankA1)

D-Glucose Concentration (g/L) = AG x 0.8637 x Dilution Factor

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

D-Fructose Absorbance, A_F = $(A_3 – A_2)$ – (BlankA3 – BlankA2)

4. Calculate the D-Fructose concentration as follows:

D-Fructose Concentration (g/L) = A_F x 0.8694 x 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):

Repeatability r = 0.056x Reproducibility R = 0.12 + 0.076x

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

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