

ENZYMATIC ANALYSIS KIT FOR THE DETERMINATION OF GLUCOSE AND FRUCTOSE IN GRAPE JUICE AND WINE

PRODUCT

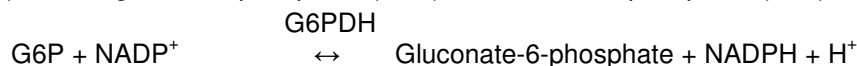
Product no. 4A140, for 30 tests, for *in vitro* use only.

PRINCIPLE OF MEASUREMENT

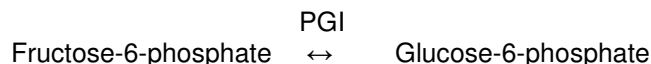
Glucose and fructose are the main sugars found in grape juice and wine and are determined enzymatically according to the following equations:



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).



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.



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 in the sample.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Add contents of Reagent No.2, Coenzymes, mix to dissolve	33 mL	1 year at 4°C 6 months once combined
2	Coenzymes (ATP/NADP)	Nil	0.2 g	1 year at 4°C
3	G6PDH/HK	Nil	0.7 mL	1 year at 4°C
4	PGI	Nil	0.7 mL	1 year at 4°C
5	Standard	Nil	3.3 mL	1 year at 4°C

The shelf life of Reagent 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 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	3.04 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 1.0 g/L. For the majority of dry wine samples, a 1 in 10 dilution is satisfactory. Semi-sweet wines may require a 1 in 20 or 1 in 50 dilution, while 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	Standard	Sample
1. Buffer	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
Sample/Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

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

c. Pipette the following reagent into the cuvettes:

3. G6PDH/HK	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)
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d. Mix well and read absorbances, A_2 , after 10 minutes.

e. Pipette the following reagent into the cuvettes:

4. PGI	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)
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f. Mix well and read absorbances, A_3 , after 10 minutes.

CALCULATIONS*

1. Calculate the Absorbance for the Sample for Glucose:

$$\text{Glucose Absorbance, } A_G = (A_2 - A_1) - (\text{Blank}A_2 - \text{Blank}A_1)$$

2. Calculate the Glucose concentration as follows;

$$\text{Glucose Concentration (g/L)} = A_G \times 0.8637 \times \text{Dilution Factor}$$

3. Do the same for the Standard by substituting the Standard absorbances in place of the Sample absorbances.

4. Calculate the Absorbance for the Sample for Fructose:

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

5. Calculate the Fructose concentration as follows:

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

6. Add the Glucose and Fructose results to get the total sugar concentration

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

$$\text{Repeatability } r = 0.056x$$

$$\text{Reproducibility } R = 0.12 + 0.076x$$

*A calculation spreadsheet is available for download at www.vintessential.com/enzyme_kits.htm

REFERENCES

1. "Compendium of International Methods of Wine and Must Analysis" OIV, Vol 1, 2006, MA-E-AS311-02-GLUFRU5, p4.

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