VINTESSENTIAL LABORATORIES

32 Brasser Avenue
Dromana Victoria 3936
Australia
T +61 3 5987 2242
F +61 3 5987 3303
E info@vintessential.com.au
W www.vintessential.com.au

ABN: 60 068 057 045

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

FOR DISCRETE ANALYSERS

PRODUCT

Product no.4B140, 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:

$$\begin{array}{lcl} & \overset{HK}{} & \\ \text{Glucose + ATP} & \rightarrow & \text{Glucose-6-phosphate + ADP} \\ \text{Fructose + ATP} & \rightarrow & \text{Fructose-6-phosphate + ADP} \end{array}$$

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).

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

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 340nm and is stoichiometrically related to the amount of glucose consumed.

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

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 in the sample.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Quantity	Stability
REAGENT 1	Buffer	19.5mL x 2	6 months at 4°C
REAGENT 2	G6PDH/HK	10.5mL x 2	12 months at 4°C
REAGENT 3	PGI	10.5mL x 2	12 months at 4°C

The shelf life of Reagent 1 can be extended by placing aliquots in a freezer. Do not freeze enzyme reagents 2 & 3. Failure to store reagents at the recommended temperature will reduce their shelf life.

SAFETY

- **Wear safety glasses, Reagent R1 is mildly corrosive**
- **The reagents contain sodium azide as preservative. Do not ingest.**

PROCEDURE

Reagent Definition

Reagent	GF R1	GF R2	GF R3
Stable on board (days)	1	1	1
Alarm limit (mL)	1.0mL	0.9mL	0.9mL
Vial volume	20mL	20mL	20mL
Syringe speed	Normal	Normal	Normal

Test Definition

Test type	Photometric
Full name	Glu & Fru
Result unit	g/l
Number of decimals	2
Acceptance	Manual
Dilution 1+	9.0
Sample type	Wine, Must, Juice

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Calibration Parameters

Calibration type	Linear
Repeat time (d)	1
Points/Calibrator	Duplicate
Acceptance	Manual
Curve direction	Ascending
Type of calibrators	Separate

Calibrator	Conc. (g/l)	Dil. Ratio 1+
GF 0.00	0.000	9.0
GF 0.50	0.500	9.0
GF 2.50	2.500	9.0
GF 5.00	5.000	9.0
GF 10.00	10.000	9.0
GF 20.00	20.000	9.0

Test Flow

Reagent	Sample	Incubation	Blank	Reagent	Reagent	Incubation	End point
Reagent	Volume (µl)	Time (sec.)	Resp. min	Reagent	Reagent	Time (sec.)	Wavelength(nm)
GF R1	4	180	*	GF R2	GF R3	300	340
Volume (µl)	Disp. with		Resp. max	Volume (µl)	Volume (µl)		Side wave.
75	Water		*	40	40		NONE
Disp. With	Volume (µl)			Disp. with	Disp. with		
Water	20			Water	Water		
Volume (µl)	Wash reagent			Volume (µl)	Volume (µl)		
47	NONE			10	14		
Wash reagent				Wash reagent	Wash reagent		Meas. Type
NONE				NONE	NONE		NORMAL

REFERENCES

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