

D-GLUCOSE/FRUCTOSE

Hexokinase UV-Method

Product #:
GF-60 (30 Tests)
GF-150 (75 Tests)
GF-500 (250 Tests)

INTENDED USE

D-Glu/Fru UniTAB™ Reagent is intended for measuring the total D-glucose D-fructose concentration in wine.

KIT CONTENTS

	<u>30T</u>	<u>75T</u>	<u>250T</u>
Glu/Fru Reagent Tablet	12	30	100
Trigger Enzyme	1.3 mL	3.3 mL	2 x 5.5 mL
D-Glu Standard	1 mL	1 mL	5mL
D-Fru Standard	1 mL	1 mL	5mL

SYSTEM REQUIREMENT

Spectrophotometer should be capable of a 0-2 A range of 340 nm absorbance readings with a 1 cm lightpath.

SAMPLES

If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples.

REAGENTS

Trigger Enzyme reagent and Standards are ready to use; store tablets tightly sealed with the desiccant pack provided. Kit contents are stable through the labeled expiration date when stored at 2-8 °C.

ASSAY PREPARATION

Working Reagent, manual:

Prepare Working Reagent just prior to testing, based on the # of wine samples to be tested. Calculate the volume of WRgt needed, based on the number of blank, standard and wine samples in your assay. Dissolve each Reagent Tablet in 5 mL deionized (DI) water using clean glassware. Mix by gentle inversion. Reconstituted reagent is stable for 2 days refrigerated, allow to reach room temperature prior to assay.

TESTING PROCEDURE

1. Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.
2. Pipet Working Reagent into cuvettes.
3. Zero spectrophotometer with Reagent Blank.
4. Wait 3 minutes and read initial absorbance ($A_{INITIAL}$)
5. Gently mix the Trigger Enzyme by inversion, add 1-drop to each cuvette, mix.
6. Incubate as specified in Table, read Final Absorbance.

Pipet into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Glu Std/Sample(s)		10µL
DI water	10µL	
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read $A_{INITIAL}$ (Initial Absorbance)		
Trigger Enzyme	40 uL (1 drop)	40 uL (1 drop)
Mix and wait 20 min. Read A_{FINAL} (Final absorbance).		

The testing range is up to 8 G/L. If test result is over-range, dilute one volume of the wine with 4-volumes of deionized (or distilled) water; re-assay & multiply this test result by "5".

CALCULATIONS

Standard Method calculation of wine results is simplified by using Unitech's

1. Our online "Flex Calculator™-GF" spreadsheet at <http://unitechscientific.com/calculators.htm> is available for download. G/L values will be calculated automatically.

2. Manual Calculation:

Calculate ΔA values and G/L as follows for each cuvette:

$$\Delta A = A_{FINAL} - A_{INITIAL}$$

Subtract the ΔA of the Reagent Blank from each sample and standard the ΔA :

$$Net A = \Delta A_{SAMPLE} - \Delta A_{BLANK}$$

Calculate GF Concentration (from 3.0 G/L D-Glu Standard)

$$D-Glu/Fru G/L = (3.0) \times (d.f.) \times \frac{Net A_{SAMPLE}}{Net A_{STANDARD}}$$

QUALITY CONTROL

Include the D-Glu standard in each assay for calculating wine results (as above) by Standard Method.

If using the Alternate Calculations (Appendix), include the standard to monitor reaction completion and assess assay performance. Performance is acceptable if result of standard is within 15% of labeled value. Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micropipettors.)

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

a. **Extinction Coefficient** (results based on factor; compare standard result with known value to verify recovery.)

D-Glu/D-Fru (g/L) = $\frac{\text{Net A} \times 5.92}{\epsilon(P)(1000\text{mg/g})(SV)}$; use standard to verify recovery.

Factor is derived as follows:

$$\begin{aligned} \text{D-Glu/D-Fru (g/L)} &= \frac{\text{Net A} \times \text{MW} \times \text{T.V.} \times \text{d.f.}}{(\epsilon)(P)(1000\text{mg/g})(SV)} \\ &= \frac{\text{Net A} \times 180.16 \times 2.045 \times 1}{6.22 \times 1 \times 1000 \times 0.01} = \text{Net A} \times 5.92 \end{aligned}$$

Where:

MW = 180.16 g/mole

TV = 2.045 mL total reaction volume

d.f. = dilution factor (Ref. Note #3)

SV = 0.010 mL sample volume

ϵ (absorptivity of NAD) = 6.22 @ 334-340nm;
3.4 @ 365nm

P = 1 cm light path

Adjust calculations if alternate SV and TV are used. Sample volume inaccuracy will affect results with the extinction coefficient calculation method; use calibrated micropipettes.

b. **Multi-point standard curve** Sample concentrations are calculated from the best-fit standard curve. Standard sets available from Unitech Scientific LLC.

METHODOLOGY & CHEMICAL PRINCIPLES

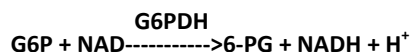
The enzyme hexokinase (HK) catalyses the phosphorylation of D-fructose and D-glucose by adenosine-5'-triphosphate (ATP). Fructose-6-phosphate (F6P) and Glucose 6 phosphate (G6P), respectively, and adenosine diphosphate (ADP), are products of these reaction.^{1,2}



F6P is converted to G6P in the presence of phosphoglucose isomerase (PGI), as follows:



In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6PDH), G6P is oxidized by nicotinamide-adenine dinucleotide (NAD); 6-phosphogluconate (6PG) and reduced nicotinamide-adenine dinucleotide (NADH) are reaction products, as shown below:



The increase in NADH concentration is measured at 340 nm and is the basis for calculating D-Glucose/Fructose concentration in the sample.

SIGNIFICANCE OF MEASUREMENTS

Reducing sugars are the predominant soluble components of soft fruits, with sucrose in low amounts. D-Glucose and D-fructose are the predominant reducing sugars in grape and other fruit juices. The ratio of glucose to fructose in mature grapes is "1", but ranges from 0.7-1.2 according to variety, maturity and fermentation conditions.

AUTOMATED TESTING 'ChemWell for Wine' *analysis is linear to 8 G/L. Prepare Working Reagent according to Manual procedure (page 1.)

Placed the Working Reagent and Trigger Enzyme in CW reagent rack.

CALCULATIONS:

'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range.

TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "UniTAB" are Trademarks of Unitech Scientific LLC

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