

# D-GLUCOSE/FRUCTOSE

New option, ChemWell *Auto-Prep* tests (refer to **NOTE**)

Product #:  
**GF-F60 (30 Tests)**  
**GF-F150 (75 Tests)**  
**GF-F500 (250 Tests)**

## Hexokinase UV-Method

### INTENDED USE

D-Glucose/Fructose FLEX-REAGENT™ is intended for measuring D-glucose and/or D-fructose in wine.

### KIT CONTENTS

	<u>30T</u>	<u>75T</u>	<u>250T</u>
G/F Buffer	20 mL	50 mL	170 mL
PGI Enzyme	0.4 mL	1 mL	3X1 mL
HK/G6PDH Enzyme	0.4 mL	1 mL	3X1 mL
D-Glucose Std., 3 G/L	1 mL	5 mL	2X5 mL
D-Fructose Std., 3 G/L	1 mL	5 mL	2X5 mL

### SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 340 nm absorbance over a 0-2 A range 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

Kit contents are ready to use are stable through the labeled expiration date when stored at 2-8 °C.

### ASSAY PREPARATION

**Working Reagent**, manual (Total GF method):

Prepare Working Reagent just prior to testing, based on the number of blank, standards and wine samples in your assay.

	<u>Per test</u>
G/F Buffer (#1)	0.67 mL
PGI Enzyme (#2)	0.010 mL
Deionized Water	<u>1.33 mL</u>
<b>Appx. Total Vol.</b>	<b>2 mL</b>

Working reagent is stable for 2-days when refrigerated; allow it to reach room temperature prior to assay.

The above Working Reagent is suitable for total residual sugar (i.e. D-Glucose plus D-Fructose). For determining D-Glucose and D-Fructose concentrations separately, refer to the Appendix.

### TESTING PROCEDURE

Pipet each solution (#1-4) into the cuvettes, as shown:

	<u>Blank</u>	<u>Standard</u>	<u>Sample(s)</u>
1. <b>D.I. Water</b>	<b>10µL</b>		
2. <b>Standard/Sample</b>		<b>10µL</b>	<b>10µL</b>
3. <b>Working Reagent</b>	<b>2.0mL*</b>	<b>2.0mL</b>	<b>2.0mL</b>

Mix cuvettes, incubate 3 minutes.

Zero spectrophotometer (340 nm) with Reagent Blank

\*Note: 2.0mL = 2000µL

Read A<sub>INITIAL</sub> (Initial ABS)

Mix HK/G6 Enzyme

4. **HK-G6PDH Solution** **10µL**    **10µL**    **10µL**

Mix cuvettes, incubate 20 minutes, Read A<sub>FINAL</sub> (Final ABS).

If any test result is higher than 8 G/L,, dilute the sample with deionized (or distilled) water; re-assay & multiply this test result by the dilution factor.

### CALCULATIONS

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:

$$\Delta A = A_2 - A_1$$

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

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

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

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

### QUALITY CONTROL

Test the D-Glucose standard in each assay for calculating wine results (as above) by Standard Method; the D-Fructose standard provided is 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.) A 5-Level D-Glucose Standard kit is available from Unitech

### APPENDIX

#### NOTES for Alternate Calculations:

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

$$\text{D-Glu/D-Fru (G/L)} = \text{Net A} \times 5.92$$

Factor is derived as follows:

$$\text{Glu/Fru (g/L)} = \frac{\text{Net A} \times \text{MW} \times \text{T.V.} \times \text{d.f.}}{(\epsilon)(P)(1000\text{mg/g})(\text{SV})}$$

Where:

MW = 180.16G/mole

TV = total reaction volume (mL)

SV = sample volume (mL), See Procedure Step 2

ε (absorptivity NADP) = 6.22 @334-340nm [=3.4 @ 365nm]

P = 1 cm light path

d.f. = dilution factor (Refer to Note 3)

$$\text{D-Glu/Fru} = \frac{\text{Net A} \times 180.16 \times 2.02}{6.22 \times 1 \times 1000 \times 0.01} = 5.85 \times \text{Net A}$$

$$\text{D-Fru} = \frac{\text{Net A} \times 180.16 \times 2.03}{6.22 \times 1 \times 1000 \times 0.01} = 5.88 \times \text{Net A}$$

Sample volume inaccuracy will affect results with the extinction coefficient calculation method; use calibrated micropipettes.

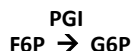
**b. Multi-point standard curve** Sample concentrations are calculated from the best-fit standard curve. Standard sets available from Unitech Scientific LLC.

## METHODOLOGY & CHEMICAL PRINCIPLES

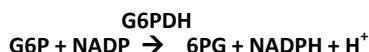
Hexokinase (HK) catalyses the phosphorylation of D-fructose and D-glucose by adenosine-5'-triphosphate (ATP). Fructose-6-phosphate (F6P) and glucose 6 phosphate (G6P), respectively, as well as adenosine diphosphate (ADP), are products of these reactions.



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



In the presence of glucose-6-phosphate dehydrogenase (G6PDH), G6P is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP); 6-phospho-gluconate (6PG) and NADPH are reaction products, as shown below:



The increase in NADPH concentration is measured at 340nm and is the basis for calculation of D-glucose and D-fructose concentration in the sample.

## SIGNIFICANCE OF MEASUREMENTS

Significance of Measurements: D-Glucose and D-fructose are the predominant reducing sugars in grape and other fruit juices (with sucrose in low amounts.) 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.

## MEASURING D-GLUCOSE & D-FRUCTOSE SEPARATELY:

Three sets of ABS readings will be taken for Reagent Blank, Glu Standard and Sample cuvettes. If desired, use D-Fru Std as known control:

**Working Reagent**, manual (Independent Glu & Fru method): **Omit PGI** when preparing the Working Reagent; otherwise prepare Working Reagent as shown on Page 1.

Pipet each solution (#1-5) into the cuvettes, as shown:

	Blank	Standard	Sample(s)
1. D.I. Water	10µL		
2. Standard/Sample		10µL	10µL
3. Working Reagent**	2.0mL	2.0mL	2.0mL

\*\*no PGI

Mix cuvettes, incubate 3 minutes.

Zero spectrophotometer (340 nm) with Reagent Blank

Read A<sub>1</sub> (Initial ABS)

Mix HK/G6 Enzyme

4. **HK-G6PDH Solution** 10µL 10µL 10µL

Mix cuvettes, incubate 20 minutes, Read A<sub>2</sub>

Mix PGI Enzyme

5. **PGI** 10µL 10µL 10µL

Mix cuvettes, incubate 20 minutes, Read A<sub>3</sub>

Calculate Net ABS values (refer to page 1).

From our "Flex Calculator" link above, select

**Glucose+Fructose (Separate Calculation) Flex Calculator** link, or use the **Standard** or **Alternate Calculation** methods above.

- D-Glu G/L: Use net A<sub>2</sub>-A<sub>1</sub> values
- D-Fru G/L: Use net A<sub>3</sub>-A<sub>2</sub> values.

## AUTOMATED TESTING - ChemWell for Wine, CW-T for Wine

**Working Reagent** is stable for 8-hours refrigerated; prepare per instructions below. Consider Unitech's [optional "AP" tests - Working Reagent preparation is automated. Refer to details: \(Optional\) AP Auto-Prep NOTE](#) below.

	# of Tests	25T	55T	90T
G/F Buffer (#1)		3mL	5mL	8mL
PGI Enzyme (#2)		0.045mL	0.075mL	0.120mL
Deionized Water		5mL	8mL	13mL
<b>WRgt</b> (Approx. Total)		8mL	13mL	21mL

(# of Tests accounts for Reagent Bottle dead volume)

Placed the **Working Reagent** and HK/G6 Enzyme in CW reagent rack.

Unitech Scientific offers AUTOMATED procedures for independent D-Glu plus D-Fru measurements; contact Unitech Scientific Tech Service.

\* **NOTE: (Optional) AP Auto-Prep ChemWell & CW-T for Wine** tests.

Simply place each kit component directly in Reagent Rack. With **AP protocols**, your CW will:

- ✓ Automatically prepare Working Reagent in each cuvette
- ✓ Optimize your reagent usage & improve lab work flow.

With Reagent Cooling and **AP protocols**, UniFLEX reagents are stable in your ChemWell reagent rack for months.

Contact Unitech Scientific Tech Service for your new **AP protocols**

## TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "FLEX Reagent" are Trademarks of Unitech Scientific

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