

D-GLUCOSE/FRUCTOSE

Hexokinase *UV-Method*

Product: G/F-F60 (30 Tests)

G/F-F150 (75 Tests)

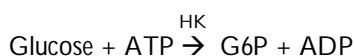
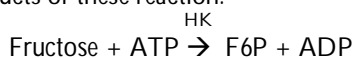
G/F-F500 (250 Tests)

INTENDED USE

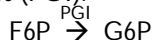
D-Glucose/Fructose FLEX-REAGENTS™ are intended for determination of D-glucose and/or D-fructose in wine, foodstuffs and other liquid samples.

METHODOLOGY & CHEMICAL PRINCIPLES

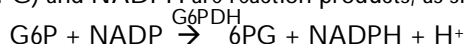
Hexokinase (HK) catalyses the phosphorylation of D-fructose and D-glucose by adenosine-5'-triphosphate (ATP). Fructose-6-phosphate (F6P) and glucose 6 phosphate (G6P), respectively, as well as adenosine diphosphate (ADP), are products of these reaction.^{1,2}



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.

REAGENTS

D-Glucose/D-Fructose FLEX-Reagent active ingredients:

	Concentration as Formulated	Quantity/Kit	
		.75T	.250T
1. <u>Glu/Fru Buffer</u>			
Triethanolamine Buffer	1 M	50 mL	170 mL
NADP	2.8 mM		
ATP	9 mM		
Mg Sulfate, Stabilizers, pH 7.6			
2. <u>PGI Suspension</u>	600 U/mL	1 mL	3 mL
3. <u>HK/G6PDH Suspension</u>		1 mL	3 mL
Hexokinase	380 U/mL		
G6PDH	190 U/mL		
4. <u>D-Glucose Standard</u>	(refer to label)	5 mL	10 mL
5. <u>D-Fructose Standard</u>		5 mL	10 mL

A 5-Level Kit of D-Glucose Standards is available from Unitech Scientific.

REAGENT PREPARATION & STORAGE

All components are ready to use; gently mix suspensions by inversion prior to use. Reagents are stable until the labeled expiration date when stored at 2-8°C.

Working Reagent: Prepare sufficient WRgt for all samples and standards in the assay, using clean glassware, according to the examples in the following tables.

1. Total D-Glucose plus D-Fructose determinations

MANUAL TESTING	2 Test	7 Tests	12 Tests	22 Tests
Glu/Fru Buffer (Bottle1)	1.33mL	5 mL	8 mL	15 mL
PGI Suspension (Bottle2)	0.020mL	0.075mL	0.120mL	0.225mL
Deionized Water	2.67mL	10 mL	16 mL	30 mL
WRgt (Approx. Total)	4 mL	15 mL	24 mL	45 mL

CHEMWELL (AUTOMATED)	25 Tests	55 Tests	90 Tests
Glu/Fru Buffer (Bottle1)	3 mL	5 mL	8 mL
PGI Suspension (Bottle2)	0.045mL	0.075mL	0.120mL
Deionized Water	5 mL	8 mL	13 mL
WRgt Volume	8 mL	13mL	21mL

(# of Tests accounts for Reagent Bottle dead volume)

2. For independent D-Glucose plus D-Fructose determinations, and alternate SV or assay parameters, refer to the Notes on Page 2.

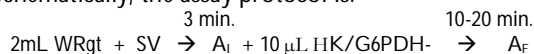
Working reagent is stable for 8 hours when refrigerated; discard any turbid working reagent or that having a 340nm absorbance greater than 0.7 when read against distilled water.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		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 ABS)		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 10-20 min. Read A _{FINAL} (Final ABS)		

System parameters: Wavelength 340 nm, Absorbance Range 0-2A, pathlength 1.0 cm.

* The 3 G/L Glucose or Fructose Standard is intended for use in this 10µL sample volume assay. The 0.5G/L Glucose Standard is intended for high sensitivity assays using 100µL sample volume (i.e. 0.03 – 0.8 G/L range). Refer to NOTES. Also note that the D-Fructose Standard is useful in monitoring the completion of the slower PGI reaction.

Schematically, the assay protocol is:



PROCEDURE

1. Allow Working Reagent (WRgt) to reach room temperature.
2. Pipet water into the Reagent Blank cuvette and pipet standards, samples into cuvettes as shown.
3. Dispense WRgt, mix and incubate 3 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read initial absorbance (A_{INITIAL}) values.
4. Gently mix the HK-G6PDH Suspension and dispense as shown above. Mix each cuvette, incubate and read the final absorbance (A_{FINAL}).

If D-Glu/D-Fru values obtained are greater than 0.8 G/L, dilute samples with deionized water
 If $A_{Final} - A_{Initial}$ (A_{F-1}) values are less than 0.1, repeat analysis with a 100 μ L sample volume or test with a less dilute sample.

CALCULATIONS

1. Calculate $A_{F-1} = A_{FINAL} - A_{INITIAL}$ for each cuvette.
2. If absorbance difference A_{F-1} for the Reagent Blank (Procedure Step 2 above) is significant, subtract this absorbance difference from that of each sample and standard.
3. Select one of the following calculation methods:
 - a. Extinction Coefficient (Use the Standard as a standard to verify recovery.)

$$G/L = \frac{A_{F-1} \times MW \times T.V. \times d.f.}{(\epsilon)(P)(1000mg/g)(SV)}$$

Where:

$$A_{F-1} = A_{FINAL} - A_{INITIAL}$$

$$MW = 180.16G/mole$$

$$TV = \text{total reaction volume (mL)}$$

$$SV = \text{sample volume (mL), See Procedure Step 2}$$

$$\epsilon \text{ (absorptivity of NADP)} = 6.22 \text{ @334-340nm [or } 3.4 \text{ @ } 365nm]$$

$$P = 1 \text{ cm light path}$$

$$d.f. = \text{dilution factor (e.g. "10" for samples diluted 1:10)}$$

10 μ L SV:

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

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

100 μ L SV:

$$D\text{-Glu or D-Glu/Fru} = \frac{A_{F-1} \times 180.16 \times 2.11}{6.22 \times 1 \times 1000 \times 0.1} = 0.611 A_{F-1}$$

$$D\text{-Fru} = \frac{A_{F-1} \times 180.16 \times 2.12}{6.22 \times 1 \times 1000 \times 0.1} = 0.614 A_{F-1}$$

Sample volume inaccuracy will affect results with this calculation method; use calibrated micropipettes.

- b. A single point standard, e.g. 3.0 G/L D-Glucose.

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

- c. A multi-point standard curve run with each assay. Sample concentrations are calculated from the best-fit standard curve.

SAMPLES

Significance of Measurements: Reducing sugars are the predominant soluble components of soft fruits, with sucrose in low amounts.³ 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 0.74-1.12 according to variety, maturity and fermentation conditions.^{4,5}

Clarification: Turbid samples should be filtered. Creep reactions occasionally occur as a result of enzymes or pigments in the sample interfering with the enzymatic reactions. Fermentation samples may be clarified by centrifugation (if necessary) and placed into a water bath at 80°C to inactivate fermentation enzymes.

Decolorization: If an unacceptably high sample blank

absorbance is obtained, mix 10 mL juice and approximately 0.1G polyamide powder or polyvinyl-polypyrrolidone (PVPP), stir for 1 minute and filter. Red wine typically needs decolorization only when SV larger than 100 μ L are used.

QUALITY CONTROL

The D-Fructose standard provided may be included in each set of assays to monitor reaction completion (completion is limited by PGI reaction rate) and control for sample dilution accuracy. Factors that may affect the performance of this test include proper instrument function, temperature standard, glassware cleanliness, and pipetting accuracy.

NOTES:

1. Wavelength: NADPH absorbance maximum is 340nm; 334-340nm determinations provides the best analytical discrimination. While less sensitive, 365nm provides a broader measuring range, e.g. 0.3 - 8 g/L @ 334-340nm vs. 0.5 - 12 @ 365nm for 10 μ L/2mL.
2. SV, High Sensitivity & Working Reagent Preparation: Assay sensitivity increases with higher SV's. For SV \geq 100 μ L, reduce water so that total "DI water + SV" is between 2 and 2.1 mL.

GLU/FRU, G/L		SV	D.I Water
334-340nm ¹	365nm		per mL Buffer
0.3 - 8	0.5 - 12	10 μ L	2.0 mL
0.1 - 2.5	0.17 - 4	30 μ L	2.0 mL
0.03 - 0.8	0.05 - 1.2	100 μ L	2.0 mL
0.006 - 0.13	0.01 - 0.2	500 μ L	1.5 mL
0.002 - 0.04	0.003 - 0.09	2000 μ L	none

Select standards within the linear assay range.

3. Sample Dilution:

Estimated D-GLU/D-FRU	Dilution
\geq 80 G/L must, dessert wines	1:100
8 to 80 G/L sweet wines	1:10
< 8.0 G/L medium and dry wines	neat

Multiply the result by the dilution factor, e.g., when diluting 1 part sample + 9 parts D.I. water, the dilution factor is "10".

4. Independent D-Glucose plus D-Fructose Values: Omit PGI from the WRgt. Follow Procedure (p. 1); calculate D-Glu from A2-A1. Immediately after the 1st 10-20' incubation, add 10 μ L PGI/cuvette, incubate 10-20', and calculate D-Fru from A3-A2.

REFERENCES

1. Barthelmai, W, and R Czek, Lin. Wochenscht, 40:585 (1962).
2. A proposed Method for determining Glucose Using Hexokinase and Glucose-6-Phosphate Dehydrog-enase, Public Health Service, Center for Disease Standard, (1976).
- 3.Green, A, in "Biochemistry of fruits and their Products," Vol 2, Ch 11, AC Hulme, ed., Academic, London and New York, 1971.
4. Amerine, MA, Thoukis, G. Vitis (1958) 1, 224.
5. Kliewer, WM, Amer. J. Enol Viticult (1965) 18, 87.

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