

D-GLUCOSE/D-FRUCTOSE

Hexokinase UV Method

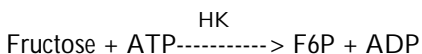
Product # GF-60 (30 Tests)
GF-150 (75 Tests) GF-500 (250 Tests)

INTENDED USE

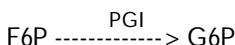
Unitech Scientific D-Glucose/D-Fructose Reagents are intended for simultaneous determination of D-glucose/D-fructose in wine, foodstuffs and other liquid samples.

METHODOLOGY & CHEMICAL PRINCIPLES

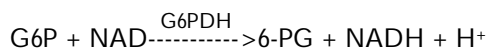
The enzyme hexokinase (HK) catalyses the phosphorylation of D-fructose and D-glucose by adenosine-5'-triphosphate (ATP). Fructose-6-phosphate (F6P) and Glucose 6 phosphate (G6P), respectively, and adenosine diphosphate (ADP), are products of these reaction.^{1,2}



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 nicotin-amide-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.

REAGENTS

Active Ingredients are:

Glu/Fru Reagent Tablets	Concentration as Formulated	Quantity/Kit	
		75T	250T
Hexokinase	1.5 KU/L	30	100
ATP	1.3 mM		
NAD	0.65 mM		
Buffer pH 7.6	50 mM		
<u>Trigger Enzyme</u>		3.5mL	11.6mL
PGI	200 U/L		
G6PDH	1.25 KU/L		
NH ₄ Sulfate	3.2M		
<u>D-Glu Standard</u>	3.0 g/L	1 mL	5 mL
<u>D-Fru Standard</u>	3.0 g/L	1 mL	5 mL

STORAGE & REAGENT PREPARATION

Components are stable until the labeled expiration date when stored in their original container at 2-8°C; store tablets tightly sealed with the desiccant pack provided. Trigger Enzyme reagent and Standards are ready to use and require no reconstitution.

Working Reagent (WRgt)

Calculate the volume of WRgt required for all samples and standards in the assay. Dissolve each Reagent Tablet in 5 mL deionized (DI) water using clean glassware. Mix by gentle inversion. For high sensitivity assays, refer to NOTES.

Reconstituted reagent is stable for 7 days at 2-8°C. Discard any turbid reagent or that with absorbance greater than 0.30, 340 nm read against a deionized water blank.

PROCEDURE

System parameters: Wavelength 340 nm, absorbance range 0-2A, pathlength 1.0 cm. For alternative sample volumes (SV), wavelengths, etc, refer to NOTES.

Pipet into Cuvettes	Reagent Blank Cuvette	Sample or Standard 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 Absorbance)		
Trigger Enzyme	35 µL (1 drop)	35 µL (1 drop)
Mix and wait 10-20 min. Read A _{FINAL} (Final absorbance).		

1. Label one cuvette for each sample, standard, and blank.
2. Prepare sufficient working WRgt. Allow WRgt to reach room temperature.
3. Pipet standards, samples, and water into cuvettes as shown in the Table above using calibrated pipets.
4. Dispense WRgt into each cuvette, mix and wait 3 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read initial absorbance (A_{INITIAL}) values.
5. Gently mix the Trigger Enzyme by inversion and dispense, as shown above. Mix each cuvette, wait 10-20 min. and read the final absorbance (A_{FINAL}).

CALCULATIONS

1. Calculate Delta A = A_{FINAL} - A_{INITIAL} for each cuvette.
2. If the Delta A for the Reagent Blank is significant, subtract it from each sample and standard Delta A.:
Net A = Delta A_{SAMPLE} - Delta A_{BLANK}
3. Samples with Delta A values < 0.05 should be reassayed with a larger sample volume or at a lower dilution.

4. Select one of the following calculation methods:
 a. Extinction Coefficient (Use standard to verify recovery.)

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

$$= \frac{\text{Net A} \times 180.16 \times 2.045}{6.22 \times 1 \times 1000 \times 0.01} = \text{Net A} \times 5.92$$

Where:

MW = 180.16 g/mole

TV = 2.045 mL total reaction volume

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 or if diluted samples are reassayed; e.g. if SV = 100 μ L, the Extinction Coefficient calculation is:

$$D\text{-Glu/D-Fru} = \frac{\text{Net A} \times 180.16 \times 2.135}{6.22 \times 1 \times 1000 \times 0.100}$$

$$= \text{Net A} \times 0.618$$

Note that calibration accuracy of pipets affects results with extinction coefficient calculations.

- b. A Single-point Standard, e.g. 3.0 g/L D-Glucose.
 D-Glu/D-Fru, g/L = Conc. Standard x $\frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$
 $= 3.0 \times \frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$
- c. A Multi-point Standard (e.g. Unitech 5-Point Standards) Sample concentrations are calculated from the best-fit standard curve.

NOTES

1. Wavelength:
 NADH absorbance maximum is 340nm; 334-340nm determinations provide the best sensitivity. Alternatively, 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 and High Sensitivity Assays:
 Sensitivity increases with higher SV's. For SV \geq 100 μ L, dissolve each tablet in a reduced volume of water to maintain reactant concentrations:

GLU/FRU, g/L		D.I Water per Tablet	
<u>334-340nm</u>	<u>365nm</u>	<u>SV</u>	<u>per Tablet</u>
0.3 - 8	0.5 - 12	10 μ L	5.0 mL
0.03 - 0.8	0.05 - 1.2	100 μ L	5.0 mL
0.006 - 0.13	0.01 - 0.2	500 μ L	3.0 mL

3. Linearity and Sample Dilution:
 Results are linear to 8 g/L. Dilute samples with A_{Final} values near instrumentation absorbance limits (typically 3.0 O.D.) with deionized water and reassay:

<u>Estimated D-GLU/D-FRU</u>	<u>Dilution</u>
\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 calculated g/L result by the dilution factor.
 e.g. when diluting 1 part sample with 9 parts deionized water, the dilution factor is "10".

4. Creep reactions occasionally occur as a result of enzymes or pigments in the sample interfering with the enzymatic reactions. If necessary, prepare a sample blank, i.e. prepare two tubes:
 a. Sample Blank [Rgt + Sample]
 b. Reaction [Rgt + Sample + Trigger]
 Calculate corrected Delta A =
 $\text{Delta A}_{\text{REACTION}} - \text{Delta A}_{\text{BLANK}}$
 Use this corrected Delta A to calculate results.

SAMPLE PREPARATION

Clarification

Turbid samples should be filtered. Fermentation samples may be clarified by centrifugation (if necessary) and placed into a water bath at 80°C to inactivate fermentation enzymes.

Decolorization

Red wine typically needs decolorization only when SV larger than 100 μ L are used. If an unacceptably high sample blank absorbance is obtained, mix 10 mL juice and approximately 0.1 g polyamide powder or polyvinylpoly-pyrrolidone (PVPP), stir for 1 minute and filter.

SIGNIFICANCE OF MEASUREMENTS

Reducing sugars are the predominant soluble components of soft fruits. D-glucose and D-fructose are the predominant reducing sugars in grape and other fruit juices, with sucrose present in low amounts³. 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}

QUALITY CONTROL

Low and high level controls should be included in each set of assays. The Fructose dehydrogenase reaction depends on PGI and proceeds more slowly than that of Glucose; use the D-Fructose Standard to assess reaction completeness. It is expected that the labeled value of standards will be recovered in the assay. Factors that may affect the performance of this test include proper instrument function, temperature control, glassware cleanliness, and pipetting accuracy.

REFERENCES

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