

D-GLUCOSE

Hexokinase *UV-Method*

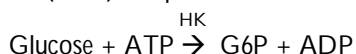
Product: GLU-F60 (30 Tests)
GLU-F150 (75 Tests)

INTENDED USE

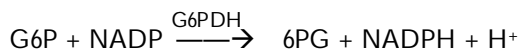
D-Glucose FLEX-REAGENTS™ are intended for determination of D-glucose in wine, foodstuffs and other liquid samples.

METHODOLOGY & CHEMICAL PRINCIPLES

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



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 concentration in the sample.

REAGENTS

D-Glucose FLEX-Reagent active ingredients:

| | Concentration as Formulated | Quantity/Kit | 75T | 250T |
|---------------------------------|-----------------------------|--------------|--------|------|
| 1. <u>Glucose/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>HK/G6PDH Suspension</u> | | 1 mL | 3 mL | |
| Hexokinase | 380 U/mL | | | |
| G6PDH | 190 U/mL | | | |
| 3. <u>D-Glucose Standard</u> | (refer to label) | 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. D-Glucose determinations

| MANUAL TESTING | 2 Tests | 7 Tests | 12 Tests | 22 Tests |
|------------------------------|-------------|--------------|--------------|--------------|
| Glucose/Fru Buffer (Bottle1) | 1.33mL | 5 mL | 8 mL | 15 mL |
| 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 |
|--|----------|----------|----------|
| Glucose/Fru Buffer (Bottle1) | 3 mL | 5 mL | 8 mL |
| Deionized Water | 5 mL | 8 mL | 13 mL |
| WRgt Volume | 8 mL | 13 mL | 21 mL |
| (# of Tests accounts for Reagent Bottle dead volume) | | | |

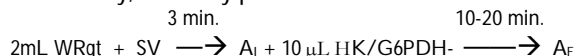
Working reagent is stable for 8 hours when refrigerated; discard any turbid working reagent or that having a 340nm absorbance greater than 0.7 when read against distilled water.

| 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 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.

Schematically, the assay protocol is:



PROCEDURE

- Allow Working Reagent (WRgt) to reach room temperature.
- Pipet water into the Reagent Blank cuvette and pipet standards, samples into cuvettes as shown.
- Dispense WRgt, mix and incubate 3 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read initial absorbance (A_{INITIAL}) values.
- 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 values obtained are greater than 0.8 G/L, dilute samples with deionized water

If A_{Final} - Initial (A_{F-I}) values are less than 0.1, repeat analysis with a 100µL sample volume or test with a less dilute sample.

CALCULATIONS

- Calculate A_{F-I} = A_{FINAL} - A_{INITIAL} for each cuvette.
- If absorbance difference A_{F-I} 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.)

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

Where:

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

$$MW = 180.16\text{G/mole}$$

TV = total reaction volume (mL)

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

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

P = 1 cm light path

d.f. = dilution factor (e.g. "10" for samples diluted 1:10)

10 μ L SV:

$$D\text{-Glucose} = \frac{A_{F-1} \times 180.16 \times 2.02}{6.22 \times 1 \times 1000 \times 0.01} = 5.85A_{F-1}$$

100 μ L SV:

$$D\text{-Glucose} = \frac{A_{F-1} \times 180.16 \times 2.11}{6.22 \times 1 \times 1000 \times 0.1} = 0.611 A_{F-1}$$

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.

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

- 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-polyrrolidone (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-Glucose standard provided may be included in each set of assays to monitor reaction completion +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:

- 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.
- SV, High Sensitivity & Working Reagent Preparation: Assay sensitivity increases with higher SV's. For SV \geq 100 μ L, reduce water so that total "D.I water + SV" is between 2 and 2.1 mL.

| GLU G/L | | SV | D.I Water per mL Buffer |
|------------------------|--------------|--------------|-------------------------|
| 334-340nm ¹ | 365nm | | |
| 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 | 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".

REFERENCES

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

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