UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

D-GLUCOSE

Hexokinase UV-Method

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

INTENDED USE

D-Glucose FLEX-REAGENT™ is intended for measuring D-glucose concentration in wine.

KIT CONTENTS

	30T	75T	250T
G/F Buffer	20 mL	50 mL	170 mL
HK/G6PDH Suspension	0.4 mL	1 mL	3X1 mL
D-Glucose 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:
(see Appendix for ChemWell for Wine™ **Working Reagent**)
Prepare Working Reagent just prior to testing, based on the number of blank, standards and wine samples in your assay.

	Per test
G/F Buffer (#1)	0.67 mL
Deionized Water	1.33 mL
Appx. Total Vol.	**2 mL**

Working reagent is stable for 2-days when refrigerated; allow it 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 into cuvettes.
3. Zero spectrophotometer with Reagent Blank.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
DI water	10µL	
Glu Std / Sample(s)		10µL
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read $A_{INITIAL}$ (Initial ABS) at 340 nm		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 20 min. Read A_{FINAL} (Final ABS)		

4. Wait 3 minutes and read initial absorbance ($A_{INITIAL}$)
5. Gently mix HK/G6 Enzyme by inversion and pipet into cuvettes, mix.
6. Incubate as specified in Table, read Final Absorbance.

The testing range is up to 8 G/L. If test result is over-range, 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:

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

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

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

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

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

QUALITY CONTROL

Test the D-Glucose standard in each assay for calculating wine results (as above) by Standard Method. A standard may be run as a known sample; 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.)
D-Glu (G/L) = Net A X 5.92)

Factor is derived as follows:

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

Where:

MW =180.16G/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 (Refer to Note 3)

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

Sample volume inaccuracy will affect results with the extinction coeficient 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-glucose by adenosine-5'-triphosphate (ATP). Glucose 6 phosphate (G6P) and adenosine diphosphate (ADP) are products of these reactions.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G6P} + \text{ADP}$$

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:

$$\text{G6P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{6PG} + \text{NADPH} + \text{H}^+$$

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

SIGNIFICANCE OF MEASUREMENTS

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' Glu analysis is linear to 8 G/L and uses the following **Working Reagent,** which is stable for 8-hours refrigerated.

(# of Tests accounts for Reagent Bottle dead volume)

Placed the **Working Reagent** and HK/G6 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.

# of Tests	**25T**	**55T**	**90T**
G/F Buffer (#1)	3mL	5mL	8mL
Deionized Water	5mL	8mL	13mL
WRgt (Approx. Total)	**8mL**	**13mL**	**21mL**

MANUFACTURED BY: **UNITECH SCIENTIFIC**
12026 Centralia Road Suite H, Hawaiian Gardens, CA 90716
Tel: 562-924-5150 Fax: 562-809-3140
www.unitechscientific.com