

### SUCROSE Invertase hydrolysis Reagent (for Total D-GLU/FRU Determination)

*Invertase UV-Method*

Product #: Suc-80 (80 tests)

*(Required, sold separately: [GF-UniFLEX Reagent](#))*

#### INTENDED USE

For Sucrose determination in wine; used in conjunction with Unitech's UniFLEX GF Reagent Kit to determine Sucrose from Total (hydrolyzed) D-Glucose/Fructose, then subtracting the measured D-Glucose/D-Fructose concentrations.

#### KIT CONTENTS

Sucrose Invertase 20 mL  
Sucrose Std., 5 G/L 2.5 mL

#### SAMPLE

Wine may be tested directly. Degass Sparkling wine. Filter or centrifuge Turbid samples.

#### REAGENTS

Components of the kit:

**Invertase Enzyme Suspension** (80 Tests), 20 mL

**Standard, 5.0 g/L Sucrose** solution, 2.5 mL

Enzyme and Standard are ready to use.

**STABILITY:** the reagents, at 2-8°C, are stable up to the expiry date shown on the package if not contaminated during handling.

**Required but not supplied:** GF-UniFLEX Reagent kit

#### UniFLEX GF ASSAY PREPARATION

Refer to Unitech GF Flex Directions for details to prepare Manual or ChemWell for Wine™ **Working Reagent**

**Prepare Working Reagent sufficient for testing two sets**

of wine samples, the first for the G/F assay to determine the 'Endogenous GF' of (untreated) samples [plus Blank, & Glu Standard]; the second to determine 'Total GF' (hydrolyzed wines and Sucrose Standard i.e. **Sucrose + Glucose + Fructose**) [plus Blank]

Let reagents reach the working temperature before using.

Perform two independent analyses as follows:

#### TESTING PROCEDURE

• Temperature: 37°C

1) Determine G/F concentrations of (untreated) wine samples.

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 <sub>INITIAL</sub> (Initial ABS) at 340 nm		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 20 min. Read A <sub>FINAL</sub> (Final ABS)		

a) Pipet water (Reagent Blank), Glu Standard, and (untreated) wine samples into respective cuvettes. Pipet Working into cuvettes.

b) Zero spectrophotometer with Reagent Blank. Wait 3 minutes and read initial absorbance (A<sub>INITIAL</sub>)

c) Gently mix HK/G6 Enzyme by inversion and pipet into cuvettes, mix, Incubate and read Final Absorbance.

The GF 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.

2) Determine 'Total GF' concentration of hydrolyzed samples

a) Complete the Hydrolysis procedure. Pipette Sucrose Standard and Wine sample(s) in labelled test tubes:

Tubes:	Standard	Samples
Invertase Reagent	250 µL	250 µL
Sucrose Standard	5 µL	---
Samples	---	5 µL

Mix and incubate for 20 minutes at 37°C (30 min. at 20C.)

b) Using the **10-fold increased Volume** shown below, pipet water, **HYDROLYZED Sucrose Std & Samples** into cuvettes. Pipet Working into cuvettes.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
DI water	100µL	
Sucrose Std / Sample(s)		100µL
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read A <sub>INITIAL</sub> (Initial ABS) at 340 nm		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 20 min. Read A <sub>FINAL</sub> (Final ABS)		

c) Zero spectrophotometer with Reagent Blank. Wait 3 minutes and read initial absorbance (A<sub>INITIAL</sub>)

d) Gently mix HK/G6 Enzyme by inversion and pipet into cuvettes, mix, Incubate and read Final Absorbance.

The Total GF testing range is up to 25 g/L. If necessary, dilute wine, re-assay & multiply this test result by dilution factor.

#### CALCULATIONS

**'Endogenous GF' CALCULATION (Per UniFLEX GF Directions,** based on results for UNTREATED SAMPLES, test Procedure 1.

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:

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

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)**

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

**'Total GF' CALCULATION**

Calculate **Total GF concentration** (i.e. Sucrose + Glucose + Fructose) based on results for HYDROLYZED SAMPLES, test

**Procedure 2.**

Calculate  $\Delta A$  values and G/L as follows for each cuvette:

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

Subtract the  $\Delta A$  of the Reagent Blank from each sample and standard the  $\Delta A$ :

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

Calculate **Total GF** - based on 5.0 G/L hydrolyzed Sucrose Standard (note that no dilution factor is required since Standard and Samples are treated the same):

$$\text{Total GF (G/L)} = (5.0) \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}}$$

**SUCROSE CALCULATION**

Calculate the initial Sucrose concentration in the sample:

$$\text{SUCROSE} = \text{'Total GF'} - \text{'Endogenous GF'}$$

**PRINCIPLE**

According to the reaction per GF-UniFLEX Reagent kit: in the presence of ATP, NADP, G6PDH (glucose-6P dehydrogenase) HK (hexokinase) and PGI, endogenous D-Glucose and D-Fructose present in the sample react causing the reduction of NADP to NADPH+ H+. NADPH+ concentration, and hence the Absorbance at 340nm wavelength, is proportional to the total glucose and fructose (fermentable sugars) present in the sample.

In a second distinct reaction, Sucrose is hydrolyzed into glucose and fructose by the enzyme invertase:

INVERTASE

Sucrose ----->D-glucose + D-fructose

The total (hydrolyzed plus endogenous) glucose plus fructose is again determined; the initial Sucrose concentration is calculated.

**UNITECH SCIENTIFIC**

12026 Centralia Road Suite H, Hawaiian Gardens, CA 90716

Tel: 562-924-5150 Fax: 562-809-3140

www.unitechscientific.com