UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

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 concentratons.

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 contamined 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 $A_{INITIAL}$ (Initial ABS) at 340 nm		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 20 min. Read A_{FINAL} (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 (AINITIAL)
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 $A_{INITIAL}$ (Initial ABS) at 340 nm		
HK-G6PDH Suspension	10 µL	10 µL
Mix and incubate 20 min. Read A_{FINAL} (Final ABS)		

c) Zero spectrophotometer with Reagent Blank. Wait 3 minutes and read initial absorbance ($A_{INITIAL}$)
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:

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

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

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

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

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

'Total GF' CALCULATION

Calculate **Total GF concentration** (i.e. Sucrose + Glucose + Fructose) based on results for HYDROLYZED SAMPLES, test **Procedure 2.**

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 **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):

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

Where the Sucrose (as Total GF) Concentration is 5G/L:

$$\textbf{Total GF (G/L)} = (25) \times \frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STANDARD}}$$

SUCROSE CALCULATION

Calculate the initial Sucrose concentration in the sample:

SUCROSE = 'Total GF'- '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.

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