UNITECH SCIENTIFIC

FLEX-REAGENT™

FREE SULFITES

Photometric method for free Sulfites in wine

Caution:Corrosive, Acid

Product #: **SO2 Free 60 (30 Tests)**
SO2 Free 150 (75 Tests)

INTENDED USE

This reagent is intended for spectrophotometric measurement of free sulfite (SO2) concentrations in wine.

KIT CONTENTS	30T	75T
Diluent	100 mL	2x125 mL
Oxidizer	2.0 mL	2.0 mL
Chromogen	10 mL	25 mL
Starter	10 mL	25 mL
20mg/L Sulfite Std	5 mL	5 mL

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 545 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 such, e.g. juice, must or fermentation samples.

REAGENTS

The reagents supplied are ready-to-use; in the original, tightly closed containers. Kit contents are stable through the labelled expiration date when stored between 2-8° C. Protect the Chromogen from direct light. Crystals may form which do NOT affect reagent performance.

WARNINGS The Oxidizer and Chromogen are corrosive. Wear suitable eye protection and gloves; in case of eye contact, rinse immediately with plenty of water, seek medical attention. Dispose of reagents into sink followed by water (i.e. per local regulations.)

ASSAY PREPARATION

Working Blank & Working Reagent

Prepare Working Blank (W-BL) & Working Reagent (**W-Rgt**) using the table as a guide. Mix solution prior to use. (Our "Flex Calculator™" spreadsheet will calculate component volumes for your assay size; download this spreadsheet at *http://www.unitechscientific.com/prodlit.htm#calculators*.)

REAGENTS	6 Cuvettes		15 Cuvettes	
	W-BL	W-Rgt	W-BL	W-Rgt
1. Diluent	10mL	10mL	25mL	25mL
2. Oxidizer	0.10mL Mix *	-	0.25mL Mix *	-
3. Chromogen	1mL	1mL	2.5mL	2.5mL
4. Starter	1mL	1mL	2.5mL	2.5mL
Total mL	*12.1mL*	*12mL*	*30.2mL*	*30mL*

* **Critical Note** regarding preparation of **Working Blank Solution: MIX** Diluent(1) with Oxidizer(2) prior to adding Chromogen(3) and Starter(4).

NOTES & PRECAUTIONS Store kit components in their original containers; crystals may form (which do NOT affect reagent performance.) Use only clean pipettes and containers.

Working Reagents (**W-BL** & **W-Rgt**) are stable for 2-days at 5C. Refrigerate for 15-minutes prior to assay and keep refrigerated until ready to begin assay.

PROCEDURE

1. Two Zero-Rgt Cuvettes will be used to zero the spec. Label a Zero-Rgt Blank & Reaction Cuvette. Pipet 50uL DI Water into each of these cuvettes, as shown in the table.
2. For each Standard and each Sample, label a Blank & Reaction Cuvette. Pipet each Standard & Sample into both (BL & Rx) sets of cuvettes, as shown in the table.

	Volume/Cuvette					
	Sample Blank Cuvettes			Reaction Cuvettes		
ADD	*Rgt Blank*	*Standard*	*Samples*	*Rgt Blank*	*Standard*	*Samples*
DI Water	*50uL*			*50uL*		
Standard or Sample		*50uL*	*50uL*		*50uL*	*50uL*
Working Blank	2000uL					
Working Reagent				2000uL		
Mix, keep cool for 4-min., Zero Spec & **Read Blank Cuvettes** - *refer to Step #4* Zero Spec & **Read Reaction Cuvettes** - *refer to Step #5*						
Zero & Read ABS_{545nm}	**A = 0** *"Zero* **Rgt Blank**	$A_{SAMPLE-BL}$ **Read Blank Cuvettes**		**A = 0** *"Zero* **Rgt Blank**	A_{SAMPLE} **Read Reaction Cuvettes**	

3. Dispense chilled W-BL and W-Rgt solutions to respective cuvettes (refer to Table) and incubate 4 minutes.
4. Start with the **Sample Blank** set of cuvettes: Zero the Spec with **Blank Cuvette**; read each Standard & Sample cuvette.
5. Immediately continue with the **Reaction** set of tubes: Re-zero Spec with Reagent Blank cuvette; read each Standard & Sample cuvette.

The testing range is up to 100 mG/L (=ppm). If test result is over-range, dilute sample and retest; multiply result by dilution factor.

CALCULATIONS

Calculation of Sulfite concentrations is simplified by using Unitech's

1. Online "Flex Calculator™-SO2" spreadsheet at *http://unitechscientific.com/calculators.htm* is available for download, mG/L values will be calculated automatically.
2. Manual Calculation:
   Calculate Net ABS values by subtracting corresponding Blank ABS values from Reaction ABS values:
   Net A_{STD} = A_{STD} - A_{STD-BL}
   Net A_{SAMPLE} = A_{SAMPLE} - $A_{SAMPLE-BL}$

Calculate Free Sulfite concentrations from the standard provided:

Free Sulfites (SO_2), mg/L (ppm) = 20 X $\frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STD}}$

QUALITY CONTROL

Include the Sulfite standard in each assay for calculating wine results (as above) by Standard Method.

Include a Check Wine with known Sulfite concentration (or alternatively an aqueous Sulfite Standard) in each assay tp monitor assay performance. Performance is acceptable if result of 'Check Sample' is within 10 mG/L of the known value. Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micropipettors.)

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

Multi-point standard curve: Sample concentrations may be calculated from the best-fit standard curve. Standard sets available from Unitech Scientific LLC.

METHODOLOGY & CHEMICAL PRINCIPLES

This end-point photometric test for Free Sulfites in wine is based on the reaction of sulfites with fucsin dyes with aldehydes. In acid solutions, free sulfites react with fucsin compounds and formaldehyde to produce a magenta colored chromophor measured at 545nm. Sample blanking corrects for the absorbance from polyphenols and wine pigments. Controlling incubation time and lowering reaction temperature minimize interferrence of bound SO2 in the samples.

Terminology: Sulfur dioxide (SO_2) is present in wine both bound to components such as polyphenolics and sugars and as 'Free sulfites' (i.e. freely dissolved bisulfite ion (HSO_3^-) and molecular SO_2 [H_2SO_3]). The equilibrium of these two free forms is governed by pH and temperature: $H_2SO_3 \Leftrightarrow HSO_3^-$.

'Total sulfites' refers to the concentration of all SO2 forms (free and bound) present in wine.

AUTOMATED TESTING 'ChemWell for Wine' *analysis is linear to 100 mG/L. Placed the Working Reagent and Working Blank in CW reagent rack.

ASSAY PREPARATION

Working Blank & Working Reagent based on 0.2mL/Test

	10 CW Tests		53 CW Tests	
REAGENTS	W-BL	W-Rgt	W-BL	W-Rgt
1. Diluent	3mL	3mL	10mL	10mL
2. Oxidizer	0.03mL Mix *	-	0.10mL Mix *	-
3. Chromogen	0.3mL	0.3mL	1mL	1mL
4. Starter	0.3mL	0.3mL	1mL	1mL
Total mL	*3.6mL*	*3.6mL*	*12.1mL*	*12mL*
*mL - dead **vol***	*2mL*	*2mL*	***12.1mL***	*12mL*

'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range.

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