

UNITECH SCIENTIFIC

FLEX-REAGENT™

TOTAL SULFITES

Photometric method for total Sulfites in wine

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

INTENDED USE

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

KIT CONTENTS

	30T	75T
Color Reagent	2x30 mL	1x30 & 1x120mL
Blanking Reagent	60 mL	150 mL
50mg/L Sulfite Std	5 mL	5 mL

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 405-420 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, and are stable through the expiration date when stored between +5 to +8° C in the original, tightly closed containers.

WARNINGS These reagents are harmful. Wear suitable eye protection and gloves. In case of contact with the eyes, rinse immediately with plenty of water and seek medical attention. Dispose of unused reagents into sink, followed by fresh water (i.e. per local regulations.)

ASSAY PREPARATION

Calculate the volume needed for wine samples plus standard and blank cuvettes. Pipette the required volume of **Working Blank & Reagent** directly from the container using clean pipettes.

PRECAUTIONS Avoid reagent cross-contamination; crystals may form (which do NOT affect reagent performance.) Use only clean pipettes and Working Reagent bottles.

PROCEDURE

- 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.
- 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.
- Add **W-BL** and **W-Rgt** solutions to respective cuvettes (refer to Table) and incubate at room temperature.
- Zero the Spec with **Zero-Rgt Blank Cuvette**; read Standard & Sample **Blank** cuvettes.
- Re-zero Spec with **Zero Reaction Cuvette**; read Standard

and Sample Reaction cuvettes.

ADD	Volume/Cuvette			
	Zero-Rgt Cuvettes		Standard(s) or Sample(s)	
	Blank Cuvette	Reaction Cuvette	Blank Cuvette	Reaction Cuvette
DI Water	50µL	50µL	-	-
Standard or Sample			50µL	50µL
W-BL	2000µL	-	2000µL	-
W-Rgt	-	2000µL	-	2000µL
Mix, wait 5-minutes				
Zero Spec & Read Blank Cuvettes - refer to Step #4				
Zero Spec & Read Reaction Cuvettes - refer to Step #5				
Zero Spec & Read ABS _{420nm}	A = 0 "Zero Rgt Blank Cuvette"	A = 0 "Zero Reaction Cuvette"	A _{SAMPLE-BL}	A _{SAMPLE}

The testing range is up to 200 mg/L (=ppm). If a calculated result is over-range, dilute sample and retest; multiply this test result by the dilution factor.

CALCULATIONS

- Our online "Flex Calculator™-SO₂" spreadsheet at <http://unitechscientific.com/calculators.htm> is available for download, mg/L values will be calculated automatically.
- Manual Calculation:**

Calculate Net ABS values by subtracting corresponding Blank ABS values from Reaction ABS values:

$$\text{Net } A_{\text{STD}} = A_{\text{STD}} - A_{\text{STD-BL}}$$

$$\text{Net } A_{\text{SAMPLE}} = A_{\text{SAMPLE}} - A_{\text{SAMPLE-BL}}$$

Calculate Total Sulfite concentrations (based on the 50 mg/L Sulfite standard):

$$\text{Total Sulfites (SO}_2\text{), mg/L (ppm)} = 50 \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STD}}}$$

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 to 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 Total Sulfites in wine is based on the dissociation of bound sulfites at neutral pH and their reaction with Ellman's reagent to produce a colored reaction product which is measured photometrically at 412nm (405-420nm.) Sample blanking corrects for the absorbance from polyphenols and wine pigments.

Terminology: Sulfur dioxide (SO₂) is present in wine both bound to components such as polyphenolics and sugars and as 'Free sulfites' (i.e. freely dissolved bisulfite ion (HSO₃⁻) and molecular SO₂ [H₂SO₃]). Free and bound sulfite concentrations are in equilibrium, loosely bound sulfites become freely dissolved as the concentration of free sulfites diminish. '*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 200 mg/L. Place the Working Reagent and Working Blank in CW reagent rack.

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

TRADEMARKS: "Flex Calculator" and "FLEX Reagent" are Trademarks of Unitech Scientific LLC

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