

# UNITECH SCIENTIFIC

## FLEX-REAGENT™ TOTAL SULFITES

Product #: T-SO2 60 (30 Tests)  
T-SO2 150 (75 Tests)  
T-SO2 500 (250 Tests)

Photometric method for total Sulfites in wine

### INTENDED USE

This reagent is intended for spectrophotometric determination of total (*free + bound*) sulfite concentrations in wine.

### METHODOLOGY & CHEMICAL PRINCIPLES

Sulfur dioxide (SO<sub>2</sub>) is present in wine as both freely dissolved bisulfite ion (HSO<sub>3</sub><sup>-</sup>) and molecular SO<sub>2</sub> (H<sub>2</sub>SO<sub>3</sub>), and as sulfites bound to components such as polyphenolics and sugars. "Total Sulfites" refers to all SO<sub>2</sub> forms present in wine or must, both free and bound.

This end-point photometric test 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.

### REAGENTS & STORAGE

The reagents supplied are stable through the labeled expiration date when stored between +5 to +8° C.

#### T-SO<sub>2</sub> Kit Configuration

	30-T	75-T	250-T
1. Color Reagent	60 mL	150 mL	500 mL
2. Blanking Reagent	60 mL	150 mL	500 mL
3. 20mg/L Sulfite Std	5 mL	5 mL	5 mL

The Sulfite Standard is stable until the labeled expiration date when kept refrigerated and tightly sealed. (Remove an aliquot for use in autoanalyzers and return the Standard vial to the refrigerator.)

**PRECAUTIONS** Use only clean pipettes, avoid reagent cross-contamination. Store reagents tightly closed in original containers. Take care to use only clean pipettes, avoid cross-contamination. On automated systems, use clean WRgt bottles; avoid prolonged "topping-up" of bottles. Protect the Color Reagent (bottle 1) from direct light. Reagent turbidity indicates that reagent has deteriorated; crystals may form which do NOT affect reagent performance.

**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.)

### PROCEDURE

1. Label a Reaction and a Blank Cuvette for each standard and sample.
2. Pipette standard into both Blank & Reaction Cuvettes, pipette each sample into both Blank & Reaction Cuvettes. Dispense Blanking Reagent into the 1<sup>st</sup> set (= Blank cuvettes) and Color Reagent to the 2<sup>nd</sup> (=Reaction Cuvettes), as shown in the table below. Allow reaction to

proceed at room temperature, for 5-minutes.

3. Following the incubation zero the Spec. using the DI Water Blank Cuvette.

	Volume/Cuvettes			
	Standard(s)		Sample(s)	
	Blank Cuvettes	Reaction Cuvettes	Blank Cuvettes	Reaction Cuvettes
DI Water	50µL	50µL	50µL	50µL
Standard or Sample	50µL	50µL	50µL	50µL
Bl- Reagent	2000µL	-	2000µL	-
Color Rgt	-	2000µL	-	2000µL
<b>Mix, wait 5-min., Read Blank Cuvettes</b> (Read sample blanks at ABS <sub>412</sub> ) refer to Step #4 (Immediately) Read Reaction Cuvettes refer to Step #5				
	A <sub>STD-BL</sub>	A <sub>STD</sub>	A <sub>SAMPLE-BL</sub>	A <sub>SAMPLE</sub>

4. Read Standard and Sample Blank cuvettes.
5. Immediately, read Reaction Cuvettes (i.e. read DI Water, Standard and Sample Reaction cuvettes).

### CALCULATIONS

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 Sulfites from the standard provided:

$$\begin{aligned} \text{Total Sulfites, mg/L} &= \text{Std Conc} * \frac{\text{Net } A_{\text{Sample}}}{\text{Net } A_{\text{Std}}} \\ &= 50 * \frac{\text{Net } A_{\text{Sample}}}{\text{Net } A_{\text{Std}}} \end{aligned}$$

Alternatively, calculate Total Sulfites by a Standard Curve; standard sets are available from Unitech Scientific LLC.

This reagent is linear for total sulfites to 225 mg/L; dilute and retest sample above this range.

### QUALITY CONTROL

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

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