

UNITECH SCIENTIFIC

FLEX-REAGENT™

FREE SULFITES

Photometric method for free Sulfites in wine

Caution: Corrosive, Acid

Product #: FrSO2 F60 (30 Tests)

FrSO2 F150 (75 Tests)

FrSO2 F500 (250 Test)

INTENDED USE

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

METHODOLOGY & CHEMICAL PRINCIPLES

This end-point photometric test is based on the reaction of sulfites with fucsin dyes with aldehydes.

Terminology: 'Free sulfites' refers to sulfur dioxide (SO₂) in wine freely dissolved as bisulfite ion (HSO₃⁻) and molecular SO₂ (H₂SO₃-) - which are in equilibrium governed by pH and temperature: H₂SO₃ ⇌ HSO₃⁻.

'Total sulfites' refers to all SO₂ forms present in wine - both free and bound to wine polyphenolics, sugars, etc.

Method: In acid solutions, free sulfites react with fucsin compounds and formaldehyde to produce a magenta colored chromophore measured at 545nm. Sample blanking corrects for the absorbance from polyphenols and wine pigments. Incubation time and temperature minimize interference of bound SO₂ in the samples.¹

REAGENTS & STORAGE

The reagents supplied are stable through the expiration date (refer to product labels) when stored between +5 to +8° C in the original, tightly closed containers.

Kit Configuration

	30-T	75-T	250-T
1. Diluent	100 mL	2x125 mL	830 mL
2. Oxidizer	1.0 mL	2.0 mL	4.5 mL
3. Chromogen Conc.	10 mL	25 mL	85 mL
4. Starter	10 mL	25 mL	85 mL
5. 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. On automated systems, use clean WRgt bottles; avoid 'topping-up' bottles. Protect the Chromogen (bottle 3) from direct light. Reagent turbidity indicates that reagent has deteriorated; crystals may form which do NOT affect reagent performance.

¹ The short incubation time coupled with a reaction temperature below 15°C-20°C eliminates a potential interference; bound sulfites in wine are released during warmer incubations, falsely elevating free sulfite values – especially in darker red wines.

WARNINGS The Oxidizer (2) and Chromogen (3) are corrosive. Diluent (1), Starter (4) and Standard (5) 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 reagents into sink followed by water (i.e. per local regulations.)

REAGENT PREPARATION

Prepare both Working (W-BL, W-Rgt) Solutions using the table as a guide. Mix solution prior to use.

NOTE regarding **W-BL Solution:** **MIX** Diluent(1) with Oxidizer(2) **prior** to adding Chromogen(3) and Starter(4). Refrigerate W-BL and W-Rgt solutions for 15-minutes¹ prior to assay.

REAGENTS	6 Tests		15 Tests		30 Tests	
	W-BL	W-Rgt	W-BL	W-Rgt	W-BL	W-Rgt
1. Diluent	10	10	25	25	50	50
2. Oxidizer	0.10 mix	-	0.25 mix	-	0.50 mix	-
3. Chromogen	1	1	2.5	2.5	5	5
4. Starter	1	1	2.5	2.5	5	5
Total mL	12.1	12	30.2	30	60.5	60
# of tests, ChemWell	50		130		290	

Working Solutions (W-BL & W-Rgt) are stable for 5-days at 5C.

PROCEDURE

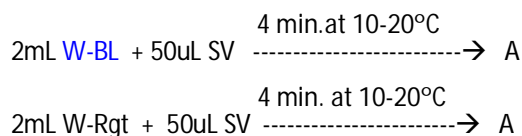
1. Label Blank & Reaction Cuvette for the Zero-Rgts, for each Standard and each Sample.

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, keep cool for 4-min., Read ABS_{545nm}				
	A = 0	A = 0	A _{SAMPLE-BL}	A _{SAMPLE}

2. Prepare Zero-Rgt Cuvettes to zero the spec by pipeting DI Water into the both BL & Rx cuvettes. Pipet each Standard & Sample into both (BL & Rx) sets of cuvettes. Add chilled W-BL and W-Rgt solutions to respective sets (refer to Table above) and incubate.

3. After timed incubation:
 - a. Zero the Spec with **Zero-Rgt Blank Cuvette**; read absorbance of each **Blank Cuvette**.
 - b. Re-zero Spec with **Zero Reaction Cuvette**; read absorbance of each Reaction Cuvette.

Schematically, the assay protocol is:



CALCULATIONS

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

$$\begin{array}{l}
 \text{Net } A_{\text{STD}} = A_{\text{STD}} - A_{\text{STD}^{\text{-BL}}} \\
 \text{Net } A_{\text{SAMPLE}} = A_{\text{SAMPLE}} - A_{\text{SAMPLE}^{\text{-BL}}}
 \end{array}$$

Calculate Free Sulfite concentrations from the standard provided:

$$\begin{aligned}
 \text{Free Sulfites (SO}_2\text{), mg/L(ppm)} &= (\text{Std Conc.}) \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STD}}} \\
 &= 20 \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STD}}}
 \end{aligned}$$

Alternatively, calculate Free Sulfites using a multi-point Standard Curve; standard sets are available from Unitech Scientific LLC.

PERFORMANCE

This reagent is specific for free sulfites and linear to 125 mg/L.

QUALITY CONTROL

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

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