

**Enzymatic Assay of SULFITE OXIDASE
(EC 1.8.3.1)**

PRINCIPLE:

Sulfite + O₂ + H₂O + Cyt c (Oxidized) Sulfite Oxidase > Sulfate + Cyt c (Reduced) + H₂O₂

Abbreviations used:

Cyt c = Cytochrome c

CONDITIONS: T = 25°C, pH = 8.5, A_{550nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 8.5 at 25°C
(Prepare 500 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.5 at 25°C with 1 M HCl.)
- B. 33 mM Sodium Sulfite Solution
(Prepare 10 ml in Reagent A using Sodium Sulfite, Anhydrous, Prod. No. S-0505.)
- C. 2 mM Cytochrome c
(Prepare 10 ml in Reagent A using Cytochrome c, from Chicken Heart, Prod. No C-0761.)
- D. Sulfite Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.07 units/ml of Sulfite Oxidase in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.77	2.77
Reagent B (Sodium Sulfite)	0.03	-----
Reagent C (Cytochrome c)	0.10	0.10
Deionized Water	-----	0.03

Equilibrate to 25°C. Monitor the $A_{550\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	0.10
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Immediately mix by inversion and record the increase in $A_{550\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{550\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{550\text{nm}}/\text{min Test} - \Delta A_{550\text{nm}}/\text{min Blank}}{(21)(\text{mg enzyme/ml RM})}$$

21 = ? Millimolar extinction coefficient between oxidized and reduced Cytochrome c at pH 8.5

RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of sulfite to sulfate during the reduction of cytochrome c per minute at pH 8.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 100 mM Tris, 0.33 mM sodium sulfite, 0.07 mM cytochrome c, and 0.005 - 0.007 unit sulfite oxidase.

REFERENCE:

MacLeod, R.M., Farkas, W., Fridovich, I. and Handler, P.

(1961) J. Biol. Chem. **236**, 1841.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.