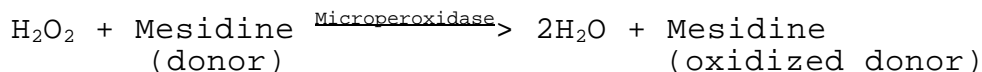


**Enzymatic Assay of MICROPEROXIDASE
Suitability Assay**

PRINCIPLE:



CONDITIONS: T = 10°C, pH = 4.9, A_{490nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 4.9 at 10°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.9 at 10°C with 1 M HCl. Store on ice.)
- B. 0.34% (w/w) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 10 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **PREPARE FRESH.** Store on ice.)
- C. 200 mM Mesidine HCl Solution (Mesidine)
(Prepare 100 ml by dissolving 2.75 g of 2,4,6-Trimethylaniline, Sigma Prod. No. T-4127 in 25 ml of 1 M HCl. Dilute to 100 ml with deionized water. Store on ice.)
- D. Microperoxidase Solution
(Immediately before use, prepare a solution containing 0.4 mg/ml of Microperoxidase in cold deionized water. Store on ice.)

PROCEDURE:

Pipette (in milliliters) the following reagents into 4 dram vials:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	7.00	7.00
Reagent B (H ₂ O ₂)	0.40	0.40
Reagent C (Mesidine)	0.60	0.60

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PROCEDURE: (Continued)

Mix by inversion and place on ice for several minutes. Place empty cuvettes into a thermostatted spectrophotometer and equilibrate to 10°C. Add 0.20 ml of Reagent D (Microperoxidase) to the Test and 0.20 ml of cold deionized water to the Blank 4 dram vials. Immediately mix by inversion and remove 3 ml from the 4 dram vials and place into the appropriate cuvettes in the spectrophotometer¹. Record the increase in A_{490nm} for approximately 20 minutes. Obtain the ΔA_{490nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\Delta A_{490\text{nm}}/\text{min}/\mu\text{M} = \frac{(\Delta A_{490\text{nm}} \text{ Test} - \Delta A_{490\text{nm}} \text{ Blank})}{\mu\text{M}}$$

$$\mu\text{M} = \frac{\text{mg/ml}}{\text{Molecular Weight}^2} \times 1000$$

mg = Weight of the Microperoxidase sample used in the assay

FINAL ASSAY CONCENTRATION:

In a 8.20 ml reaction mix, the final concentrations are 85 mM sodium acetate, 0.017% (w/w) hydrogen peroxide, 15 mM mesidine, and 0.08 mg microperoxidase.

REFERENCE:

Paul, K.-G. and Avi-Dor, Y. (1954) *Acta Chemica Scandinavica* **8**, 649-657

NOTES:

1. Ensure that no condensation forms on the cuvette walls.
2. The molecular weight of the Microperoxidase that is used in the calculation should include the water and salt content.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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