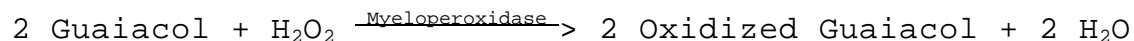


**Enzymatic Assay of MYELOPEROXIDASE
(EC 1.11.1.7)**

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 7.0, A_{470nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer with 100 mM Guaiacol and 0.0017% (w/w) Hydrogen Peroxide, pH 7.0 at 25°C (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Guaiacol, Sigma Prod. No. G-5502, and Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. Adjust to pH 7.0 at 25°C with 1 M KOH.)¹
- B. Myeloperoxidase Enzyme Solution (Immediately before use, prepare a solution containing 10 units/ml of Myeloperoxidase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	3.00	3.00

Equilibrate to 25°C. Monitor the A_{470nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized water	-----	0.035
Reagent B (Enzyme)	0.035	-----

Immediately mix by inversion. At exactly one minute record the r A_{470nm} for both the Test and Blank.

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CALCULATIONS²:

$$\text{Units/ml enzyme} = \frac{(\text{A}_{470\text{nm}} \text{ Test at 1 min} - \text{A}_{470\text{nm}} \text{ Blank at 1 min})(\text{df})}{(1.0) (0.035)}$$

df = Dilution factor

1.0 = The increase in $\text{A}_{470\text{nm}}$ /minute per unit of enzyme
(Unit Definition)

0.035 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce an increase in absorbance ($\text{A}_{470\text{nm}}$) of 1.0 per minute at pH 7.0 and 25°C, calculated from the initial rate of reaction using guaiacol as substrate.
Total reaction volume: 3.035 ml.

FINAL ASSAY CONCENTRATIONS:

In a 3.035 ml reaction mix, the final concentrations are 49 mM potassium phosphate, 99 mM guaiacol, 0.0017% (w/w) hydrogen peroxide, and 0.35 unit myeloperoxidase.

REFERENCES:

Desser, R.K., Himmelhoch, S.R., Evans, W.H., Januska, M., Mage, M., and Shelton, E. (1972) *Arch. Biochem. Biophys.*, **148**, 452-465

NOTES:

1. Guaiacol will require approximately 30 minutes of stirring for dissolution. The (w/w) concentration of the Hydrogen Peroxide is calculated by diluting the Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009.
2. The calculations are based on a final reaction mix volume of 3.035 ml. If the final volume of the reaction mix is not 3.035 ml, a correction factor must be used to determine the correct activity.
3. This assay is based on the cited reference.

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NOTES:

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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