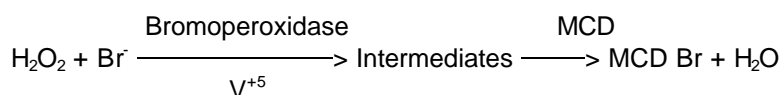


Enzymatic Assay of BROMOPEROXIDASE

PRINCIPLE:



Abbreviations used:

MCD = Monochlorodimedon

MCD = Brominated Monochlorodimedon

CONDITIONS: T = 25°C, pH = 6.4, $A_{290\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM MES Buffer, pH 6.4 at 25°C
(Prepare 250 ml in deionized water using MES, Free Acid, Hydrate, Sigma Prod. No. M-8250. Adjust to pH 6.4 at 25°C with 1 M NaOH.)
- B. 100% (v/v) Ethanol
(Use Ethyl Alcohol, Anhydrous, 200 Proof, Aldrich Stock No. 45983-6.)
- C. 0.1 mM Monochlorodimedon with 200 mM Potassium Bromide (MCD)
(Prepare by dissolving 1.2 gram of Potassium Bromide, Sigma Prod. No. P-5912 in 50 ml of Reagent A. Then dissolve 21.75 mg of Monochlorodimedon, Sigma Prod. No. M-4632 in 0.5 ml of Reagent B. Add 20 μl of the MCD solution to the KBR solution.)
- D. 100 mM Hydrogen Peroxidase (H_2O_2)
(Prepare by diluting 0.284 ml of Hydrogen Peroxidase, 30% (w/w) solution, Sigma Prod. No. H-1009 to 25 ml with Reagent C.)
- E. Bromoperoxidase Enzyme Solution
(Prepare a solution containing 0.2 mg/ml of Bromoperoxidase in Reagent A. Incubate at 25°C for 30 minutes prior to use.)

Enzymatic Assay of BROMOPEROXIDASE

Pipette (in milliliters) the following reagents into 1 ml quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent (MCD)	0.89	0.89

Incubate for 3 minutes at 25°C. Then add:

Reagent E (Enzyme Solution)	0.01	-----
Reagent A (Buffer)	-----	0.01

Mix by inversion and equilibrate to 25°C. Monitor the A_{290nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (H ₂ O ₂)	0.10	0.10
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Mix by inversion and record the decrease in A_{290nm} for several minutes. Obtain the $\Delta A_{290nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{290nm} \text{ min/Test} - \Delta A_{290nm} / \text{min Blank})(1)(df)}{(0.01)(19.9)}$$

1 = Total volume (in milliliter) of assay

df = Dilution factor

0.01 = Volume (in milliliter) of enzyme used

19.9 = Millimolar excitation coefficient of monochlorodimedon

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

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

UNIT DEFINITION:

One unit will catalyze the conversion of 1.0 micromole of monochlorodimedon to monobromochlorodimedon per min at pH 6.4 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 45 mM MES, 0.09 mM monochlorodimedon, 178 mM potassium bromide, 10 mM hydrogen peroxide, and 2 µg bromoperoxidase.

REFERENCE:

Rush, C., Willetts, A., Davies, G. Dauter, Z, Watson, H., and Littlechild, J. (1995) Febs Letters, 359, 244-246

NOTES:

1. A linear decrease of 0.3 - 0.7 absorbance units per minute should be observed.
2. This assay is based on the cited reference.
3. 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.