Enzymatic Assay of CHLOROPEROXIDASE
(EC 1.11.1.10)

PRINCIPLE:

Monochlorodimedon + H_2O_2 + Cl^- + H^+ $\xrightarrow{\text{Chloroperoxidase}}$ Dichlorodimedon + H_2O

CONDITIONS: T = 25°C, pH = 2.75, A_{278nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Citric Acid Buffer with 100 mM Potassium Phosphate, pH 2.75 at 25°C
(Prepare 200 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Prod. No. C-0759 and Potassium Phosphate, Dibasic, Prod. No. P-5504. Adjust to pH 2.75 at 25°C with 1 M HCl.)

B. 0.1 mM Monochlorodimedon with 20 mM Potassium Chloride Solution, pH 2.75 at 25°C (Monochlorodimedon)
(Prepare 100 ml in Reagent A using Monochlorodimedon, Prod. No. M-4632, and Potassium Chloride, Prod. No. P-4504. If necessary, adjust to pH 2.75 at 25°C with 1 M HCl or 1 M NaOH.)

C. 0.3% (v/v) Hydrogen Peroxide Solution
(Prepare 10 ml in deionized water using Hydrogen Peroxide, 30% (w/w), Prod. No. H-1009. **PREPARE FRESH.**)

D. Chloroperoxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 1.0 unit/ml of Chloroperoxidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent B (Monochlorodimedon)	2.90	2.90
Reagent C (H_2O_2)	0.06	0.06

Mix by inversion and equilibrate to 25°C. Monitor the A_{278nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	------	0.05
Reagent D (Enzyme Solution)	0.05	------

Immediately mix by inversion and record the decrease in A_{278nm} for approximately 5 minutes. Obtain the $r\ A_{278nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r\ A_{278nm}/\text{min Test} - r\ A_{278nm}/\text{min Blank}}{(12.2)\ (\text{mg enzyme/ml RM})}$$

12.2 = Millimolar extinction coefficient of Monochlorodimedon at 278 nm
RM = Reaction Mix

UNIT DEFINITION[1]:

One unit will catalyze the conversion of 1.0 µmole of monochlorodimedon to dichlorodimedon per minute at pH 2.75 at 25°C in the presence of potassium chloride and H_2O_2.

FINAL ASSAY CONCENTRATION:

In a 3.01 ml reaction mix, the final concentrations are 98 mM citric acid, 98 mM potassium phosphate, 0.096 mM monochlorodimedon, 19 mM potassium chloride, 0.006% (v/v) hydrogen peroxide and 0.01 - 0.05 unit of chloroperoxidase.

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REFERENCE:

P.F. Hallenberg and L.P. Hager (1978) *Methods in Enzymology*, Volume LII, Part C, 521-529.

NOTES:

1. This micromolar unit definition replaces the unit based on A_{278nm} formerly used by Sigma. One micromolar unit equals 4000 absorbance units.

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