

**Enzymatic Assay of CHLOROPEROXIDASE
(EC 1.11.1.10)**

PRINCIPLE:

Monochlorodimedon + H₂O₂ + Cl⁻ + H⁺ Chloroperoxidase > Dichlorodimedon + H₂O

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:

	<u>Test</u>	<u>Blank</u>
Reagent B (Monochlorodimedon)	2.90	2.90
Reagent C (H ₂ O 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:

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

12.2 = Millimolar extinction coefficient of
Monochlorodimedon at 278 nm

RM = Reaction Mix

UNIT DEFINITION¹:

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 H₂O₂.

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 $A_{278\text{nm}}$ 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.

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