SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of MYELOPEROXIDASE (EC 1.11.1.7)

PRINCIPLE:

2 Guaiacol + H_2O_2 $\xrightarrow{\text{Myeloperoxidase}}$ 2 Oxidized Guaiacol + 2 H_2O

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:

	Test	Blank
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 ΔA_{470nm} for both the Test and Blank.

Enzymatic Assay of MYELOPEROXIDASE
(EC 1.11.1.7)

CALCULATIONS[2]:

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

df = Dilution factor
1.0 = The increase in A_{470nm}/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 (ΔA_{470nm}) 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.

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

products, see reverse side of invoice or packing slip for additional terms and conditions of sale.