

Enzymatic Assay of LIPOXIDASE (EC 1.13.11.12)

PRINCIPLE:

Linoleic Acid + O₂ $\xrightarrow{\text{Lipoxidase}}$ Peroxide of Linoleic Acid

Abbreviation:

Peroxide of Linoleic Acid = (9Z,11E)-(13S)-13-Hydroperoxyoctadeca-9,11-dienoate

CONDITIONS: T = 25°C, pH=9.0, A_{234nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Borate Buffer, pH 9.0 at 25°C
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.0 at 25°C with 1 M NaOH.)
- B. 95% (v/v) Ethyl Alcohol (EtOH)
(Prepare 10 ml in deionized water using 200 Proof USP Ethyl Alcohol available from Quantum Chemical Company.)
- C. 0.017% (v/v) Linoleic Acid Solution (Linoleic Acid)
(Prepare by combining 0.05 ml of Reagent B and 0.05 ml of Linoleic Acid, Free Acid, Sigma Prod. No. L-1376 into a suitable container. Vortex to dissolve completely. Bring to a volume of 50 ml by slowly adding Reagent A (foaming may occur). Mix by stirring until the solution is homogeneous. Then combine 5.0 ml of the Linoleic Acid/Ethanol solution with 20 ml of Reagent A and 5.0 ml of deionized water. Mix by stirring.)
- D. Lipoxidase Enzyme Solution
(Immediately before use, prepare a solution containing 5,000 - 10,000 units/ml of Lipoxidase in Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.90	1.00
Reagent C (Linoleic Acid)	2.00	2.00

Mix by inversion and equilibrate to 25°C. Monitor the A_{234nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the increase in A_{234nm} for approximately 5 minutes. Obtain the $r A_{234nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{234nm}/\text{min Test} - r A_{234nm}/\text{min Blank})(df)}{(0.001)(0.1)}$$

df = Dilution factor

0.001 = Change in A_{234nm} per minute at pH 9.0 at 25°C when linoleic acid is the substrate in a 3.00 ml reaction volume (as per the unit definition)

0.1 = Volume (in milliliter) of enzyme used

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

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

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 178 mM boric acid, 0.011% (v/v) linoleic acid, 0.01% (v/v) ethanol and 500 - 1000 units lipoxidase.

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UNIT DEFINITION:

One unit will cause an increase in A_{234nm} of 0.001 per minute at pH 9.0 at 25EC when linoleic acid is the substrate in 3.0 ml volume (1 cm light path). One A_{234nm} unit is equivalent to the oxidation of 0.12 μ mole of linoleic acid.

REFERENCE:

Hamberg, M. and Samuelsson, B. (1967) *J. Biol. Chem.*
242, 5329

NOTES:

1. This enzyme assay is a modification of the procedure cited in the above reference.
2. 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.