Enzymatic Assay of LIPOXIDASE  
(EC 1.13.11.12)

**PRINCIPLE:**

Linoleic Acid + O$_2$ $\xrightarrow{\text{Lipoxidase}}$ 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.90</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent C (Linoleic Acid)</td>
<td>2.00</td>
<td>2.00</td>
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</table>

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 | ------ |

Immediately mix by inversion and record the increase in $A_{234nm}$ for approximately 5 minutes. Obtain the $r\ A_{234nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

$df = \text{Dilution factor}$
$0.001 = \text{Change in } A_{234nm} \text{ per minute at pH 9.0 at 25°C when linoleic acid is the substrate in a 3.00 ml reaction volume (as per the unit definition)}$
$0.1 = \text{Volume (in milliliter) of enzyme used}$

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

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

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 $A_{234\text{nm}}$ 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 $A_{234\text{nm}}$ unit is equivalent to the oxidation of 0.12 µmole of linoleic acid.

REFERENCE:


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.