Enzymatic Assay of LIPASE
(EC 3.1.1.3)
(Olive Oil as Substrate)

PRINCIPLE:

Triglyceride + H₂O → Diglyceride + Fatty Acid

CONDITIONS: T = 37°C, pH = 7.7

METHOD: Titrimetric

REAGENTS:

A. 200 mM Tris HCl Buffer, pH 7.7 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.7 at 37°C with 1 M HCl.)

B. Olive Oil Substrate Solution (Olive Oil)
   (Use Sigma Lipase Substrate, Sigma Stock No. 800-1.)

C. 95% Ethanol (Nondenatured)
   (Prepare 50 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

D. 0.9% (w/v) Thymolphthalein Indicator Solution (TPH Indic)
   (Use Thymolphthalein Indicator Solution, Sigma Stock No. 800-3, or prepare 15 ml in Reagent C using Thymolphthalein, Sigma Prod. No. T-0626.)

E. 50 mM Sodium Hydroxide Solution-Standardized (NaOH)
   (Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.)

F. Lipase Enzyme Solution
   (Immediately before use, prepare a solution containing 500 units/ml of Lipase in cold deionized water.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent B (Olive Oil)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
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<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>1.00</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by vigorously swirling and incubate at 37°C for exactly 30 minutes. Immediately after starting the incubation, pipette (in milliliters) 1.00 ml of Reagent F (Enzyme Solution) into a 50 ml Erlenmeyer flask marked "Blank" and store at 0 - 4°C.

After 30 minutes transfer the Test solution to a 50 ml Erlenmeyer flask and the Blank solution to the 50 ml Erlenmeyer flask labeled "Blank." Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (95% Ethanol)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Mix by swirling and then add 4 drops of Reagent D (TPH Indic) to both the Test and Blank solutions. Titrate each solution with Reagent E (NaOH) to a light blue color. Use a 25 ml burette with 0.1 ml graduations for the titration.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\text{NaOH})(\text{Molarity of NaOH})(1000)(2)(\text{df})}{1}
\]

(\text{NaOH}) = Volume (in milliliters) of Reagent E used for Test minus volume (in milliliters) of Reagent E used for Blank.

1000 = Conversion factor from milliequivalent to microequivalent

2 = Time conversion factor from 30 minutes to 1 hour (Unit Definition)

\text{df} = \text{Dilution factor}

1 = Volume (in milliliter) of enzyme used
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CALCULATIONS:  (continued)

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in one hour at pH 7.7 at 37°C. (This is equivalent to approximately 10 microliters of CO\textsubscript{2} in 30 minutes.)

FINAL ASSAY CONCENTRATION:

In a 7.50 ml reaction mix, the final concentrations are 26.7 mM Tris, 40% (v/v) olive oil, and 500 units lipase.

REFERENCES:


NOTES:

1. Standardization of NaOH solution is described in the cited reference.

2. All products 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.