Enzymatic Assay of LIPASE
(EC 3.1.1.3)
(Triacetin as Substrate)

PRINCIPLE:

\[ \text{Triacetin} + \text{H}_2\text{O} \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Acetic Acid} \]

CONDITIONS: \( T = 37^\circ\text{C}, \ \text{pH} = 7.4 \)

METHOD: Titrimetric

REAGENTS:

A. 150 mM Tris HCl Buffer with 330 mM Triacetin, pH 7.4 at 37°C (Buffered Triacetin)
   (Prepare 100 ml by adding 6.21 ml of Triacetin, Sigma Prod. No. T-5376 and 1.82 g of Trizma Base, Sigma Prod. No. T-1503 to 75 ml of deionized water. Adjust to pH 7.4 at 37°C with 1 M HCl and dilute to a final volume of 100 ml. Stir vigorously for approximately 30 minutes or until a homogenous dispersion is obtained. Re-adjust to pH 7.4 at 37°C with either 1 M HCl or 1 M NaOH if necessary.)

B. 95% (v/v) Ethanol
   (Prepare 20 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

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

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

E. Lipase Enzyme Solution
Immediately before use, prepare a solution containing 150 - 300 units/ml of Lipase in cold deionized water.)
Enzymatic Assay of LIPASE  
(EC 3.1.1.3)  
(Triacetin as Substrate)

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>Reagent A</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>(Buffered Triacetin)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

| Reagent E  | 1.00   | ------ |
| (Enzyme Solution) |

Mix by swirling and incubate at 37°C for exactly 60 minutes. Then add 4 drops of Reagent C (TPH Indic) to both the Test and Blank. Immediately titrate (with a graduated buret) the Test with Reagent D (NaOH) to a pale blue end point. Record the volume of Reagent D (NaOH) required. Then add:

| Reagent E  | ------ | 1.00  |
| (Enzyme Solution) |

Immediately titrate the Blank with Reagent D (NaOH) to a pale blue end point. Record the volume of Reagent D (NaOH) required.

CALCULATIONS:

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

\[
\text{NaOH} = \text{Volume (in milliliters) of Reagent D (NaOH) used for the Test minus the volume (in milliliters) of Reagent D (NaOH) used for the Blank}
\]

\[
1000 = \text{Conversion factor from milliequivalent to microequivalent}
\]

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

\[
1 = \text{Volume (in milliliter) of enzyme used in assay}
\]

UNIT DEFINITION:

One unit will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in 1 hour at pH 7.4 at 37°C.
(Incubation time: 60 minutes). (This is equivalent to approximately 10 microliters of CO₂ in 30 minutes.)
Enzymatic Assay of LIPASE
(EC 3.1.1.3)
(Triacetin as Substrate)

FINAL ASSAY CONCENTRATIONS:

In a 4.00 ml reaction mix, the final concentrations are 113 mM Tris, 248 mM triacetin, and 150 - 300 units lipase.

REFERENCE:

Sullivan, B. and Howe, M.A. (1933) *Journal of the American Chemical Society* 55, 320-324


NOTES:

1. Standardization of NaOH solution is described in (1993) *Reagent Chemicals ACS Specifications*.

2. This assay is based on the cited references.

3. 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.