

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

PRINCIPLE:



CONDITIONS: T = 37°C, 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.¹)
- E. Lipase Enzyme Solution

(Immediately before use, prepare a solution containing 150 - 300 units/ml of Lipase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffered Triacetin)	3.00	3.00

Equilibrate to 37°C. Then add:

Reagent E (Enzyme Solution)	1.00	-----
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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 (Enzyme Solution)	-----	1.00
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Immediately titrate the Blank with Reagent D (NaOH) to a pale blue end point. Record the volume of Reagent D (NaOH) required.

CALCULATIONS:

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

NaOH = 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 = Conversion factor from milliequivalent to microequivalent
df = Dilution factor
1 = 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.)

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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

(1993) *Reagent Chemicals ACS Specifications*, 8th ed., 95

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.