

**Enzymatic Assay of LIPASE
(EC 3.1.1.3)**

PRINCIPLE:

Triglyceride + H₂O $\xrightarrow{\text{Lipase}}$ Diglyceride + Fatty Acid

CONDITIONS: T = 37°C, pH = 7.7

METHOD: Titrimetric

REAGENTS:

- A. Olive Oil Substrate (Olive Oil)
(Use Sigma Lipase Substrate, Sigma Stock No. 800-1)
- B. 3000 mM Sodium Chloride Solution (NaCl)
(Prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- C. 1.5% (w/v) Sodium Taurocholate Solution (Tauro)
(Prepare 25 ml in deionized water using Taurocholic Acid, Sodium Salt, Sigma Prod. No. T-4009.)
- D. 75 mM Calcium Chloride Solution (CaCl₂)
(Prepare 25 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- E. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.¹)
- F. 5 mM Calcium Chloride Solution
(Prepare 25 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- G. Lipase Enzyme Solution
(Immediately before use, prepare a suspension containing 20,000 - 30,000 units/ml of Lipase in cold Reagent F.)

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PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Deionized Water	50.00
Reagent A (Olive Oil)	50.00
Reagent B (NaCl)	20.00
Reagent C (Tauro)	20.00
Reagent D (CaCl ₂)	10.00

Mix by swirling and adjust to pH 8.0 at 37°C with Reagent E (NaOH).

Pipette (in milliliters) the following reagents into a 50 ml beaker:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	15.00	15.00

Equilibrate to 37°C. Then add:

Reagent G (Enzyme Solution)	0.010	-----
Deionized water	-----	0.010

When the pH reaches 7.7, begin timing the reaction. Run the reaction for 1 - 5 minutes. Maintain the pH of the reaction mix at pH 7.7 by the addition of small volumes (0.025 ml) of Reagent E (NaOH). Record the volume of Reagent E (NaOH) used to maintain the pH at 7.7 and the time required.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{Molarity of NaOH})(\text{NaOH})(60)(\text{df})}{(T)(0.01)}$$

NaOH = Volume (in microliters) of Reagent E used in the Test

60 = Time conversion from minutes to hours as per the Unit Definition

df = Dilution factor

T = Time (in minutes) required to consume the added Reagent E (NaOH) while maintaining the pH at 7.7

0.01 = 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, using olive oil as substrate.

FINAL ASSAY CONCENTRATIONS:

In a 15.01 ml reaction mix, the final concentrations are 33% (v/v) olive oil, 400 mM sodium chloride, 0.2% (w/v) sodium taurocholate, 5 mM calcium chloride and 200 - 300 units lipase.

REFERENCE:

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

Worthington, C.C (1988) in *Worthington Enzyme Manual* (Worthington, C.C. ed.) 212-214, Worthington Biochemical Corporation, Freehold, NJ

NOTES:

1. The 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.