SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of LIPASE
(EC 3.1.1.3)
Sigma Prod. No. L-0763 and L-3126
(Olive Oil as Substrate)

PRINCIPLE:

Triglyceride + H_2O $\xrightarrow{\text{Lipase}}$ Diglyceride + Fatty Acid

CONDITIONS: T = 37EC, pH = 7.7

METHOD: Titrimetric

REAGENTS:

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

F. Lipase Enzyme Solution
(Immediately before use, prepare a solution containing 500 - 1,000 units/ml of Lipase in cold deionized water.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	2.50	3.50
Reagent A (Buffer)	1.00	1.00
Reagent B (Olive Oil)	3.00	3.00

Mix by swirling and equilibrate to 37EC. Then add:

Reagent F (Enzyme Solution)	1.00	------

Mix by vigorously swirling and incubate at 37EC 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 - 4EC.

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:

Reagent C (95% Ethanol)	3.00	3.00

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:

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

(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)
df = 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 37EC. (This is equivalent to approximately 10 microliters of CO_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 - 1,000 units lipase.

REFERENCES:

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

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.