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QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of LIPASE

PRINCIPLE：

Triglyceride + H_2O （Lipase）→ Diglyceride + Fatty Acid

CONDITIONS：

T = 37°C, pH = 7.2

METHOD：

Titrimetric

REAGENTS：

A. 200 mM Tris HCl Buffer, pH 7.2 at 37°C (Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 7.2 at 37°C with 1 M HCl.)

B. Olive Oil Substrate Solution (Olive Oil)

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, or prepare 15 ml in Reagent C using Thymolphthalein.)

E. 50 mM Sodium Hydroxide Solution-Standardized (NaOH) (Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous. Standardize according to the ACS Reagent Procedure. 1)

F. Lipase Enzyme Solution (Immediately before use, prepare a solution containing approximately 500 - 1000 units/ml of Lipase in cold deionized water.)

PROCEDURE：

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

	Test	Blank
Deionized Water	2.50	2.50
Reagent A (Buffer)	1.00	1.00

Reagent B (Olive Oil)	3.00	3.00
Mix by swirling and equilibrate to 37°C. Then add:		
Reagent F (Enzyme Solution)	1.00	——

Mix by 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:

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:

Units/ml enzyme =(NaOH)(Molarity of NaOH)(1000)(2)(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

Units/mg solid = （units/ml enzyme）/（ mg solid/ml enzyme）

Units/mg protein = （units/ml enzyme）/（mg protein/ml enzyme）

UNIT DEFINITION:

One unit will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in one hour at pH 7.2 at 37°C. (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.