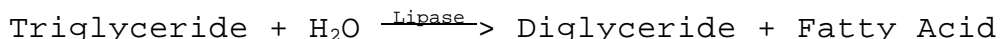


**Enzymatic Assay of LIPASE
(EC 3.1.1.3)
from Human Pancreas**

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 9.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Glycine Buffer with 19 mM Sodium Deoxycholate, pH 9.5 at 37°C (Buffer)
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126 and Deoxycholic Acid, Sodium Salt, Sigma Prod. No. D-6750. Adjust to pH 9.5 at 37°C with 1 M NaOH.)¹
- B. Ethyl Alcohol (ETOH)
(Use Ethyl Alcohol, Denatured, HPLC grade, Sigma Stock No. 27,074-1.)
- C. n-Butanol (BuOH)
(Use n-Butanol, Sigma Stock No. BT-105.)
- D. 0.3 mM Triolein Solution (Triolein)
(Prepare 101.5 ml by adding 3.5 ml of Triolein, Sigma Prod. No. T-7752 to 5 ml of Reagent C. Bring to a total volume of 100 ml using Reagent B. Then add 1.5 ml of this mixture (dropwise) to 100 ml of Reagent A.)²
- E. 0.0003% (w/v) Colipase Substrate Working Solution (CSWS)
(Prepare 101.5 ml in Reagent D using Colipase, Sigma Prod. No. C-3028.)
- F. Lipase Enzyme Solution
(Immediately before use, prepare a solution containing 325 - 650 units/ml of lipase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent E (CSWS)	2.00	2.00

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.02	-----
Deionized Water	-----	0.02

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 6 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(\text{df})}{(0.001)(0.02)}$$

df = Dilution factor

0.001 = Change in absorbance per unit as per the
Unit Definition

0.02 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will cause a change in $A_{340\text{nm}}$ of 0.001 per minute at pH 9.5 at 37°C (reaction volume = 2.02 ml).

FINAL ASSAY CONCENTRATION:

In a 2.02 ml reaction mix, the final concentrations are 98 mM glycine, 19 mM sodium deoxycholate, 0.3 mM triolein, 0.07% (v/v) n-butanol, 1.3% (v/v) ethanol, 0.0003% (w/v) colipase and 6.5 - 13 units lipase.

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REFERENCE:

Iizuka, K., Higurashi, H., Fujimoto, J., Hayashi, Y., Yamamoto, K., and Hiura, H.. (1991) *Ann. Clin. Biochem.* **28**, 373-378

NOTES:

1. Reagent A (Buffer) must be 37°C in order for the deoxycholate to dissolve. The solution should be clear and colorless.
2. Allow the triolein to sit for 25 minutes at room temperature before using. After adding the triolein to Reagent C (BuOH), allow the solution to stir for 10 - 15 minutes before adding Reagent B (ETOH). Mix by swirling for 10 - 15 minutes and continue to mix by swirling until the solution is colorless. After addition of Reagent A (Buffer) the solution will be hazy, but it should be homogenous.
3. This assay is based upon the cited reference.
4. 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.