

**Enzymatic Assay of PHOSPHOLIPASE A2
(EC 3.1.1.4)**

PRINCIPLE:

Lecithin $\xrightarrow{\text{Phospholipase A2}}$ Lysolecithin + Fatty Acid

CONDITIONS: T = 37°C, pH = 8.0

METHOD: Titrimetric

REAGENTS:

- A. 1000 mM Sodium Chloride Solution
(Prepare 100 ml in deionized water using Sodium Chloride, Prod. No. S-9625.)
- B. 100 mM Calcium Chloride Solution
(Prepare 100 ml in deionized water using Calcium Chloride, Prod. No. C-3881.)
- C. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Stock No. 505-8. Standardized according to ACS Reagent Procedure.)
- D. 2% Phosphatidylcholine Emulsion (Lecithin)
(Prepare by adding 4 grams of Phosphatidylcholine, Prod. No. P-5638, to 30 ml of Reagent A and 10 ml of Reagent B. Stir 2-3 hours at 25°C to form an emulsion. Dilute to 200 ml with deionized water. Titrate with 1 M NaOH until rate of decrease in pH is about 0.01 pH units/minute.)
- E. Phospholipase A2 Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 units/ml of Phospholipase A2 in cold deionized water.)

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PROCEDURE:

Using a suitable pH meter in conjunction with a magnetic stirrer, pipette (in milliliters) the following reagents into a suitable titration vessel:

	<u>Test</u>	<u>Blank</u>
Reagent D (Lecithin)	10.00	10.00
Adjust to pH 8.0 at 37°C. then add:		
Deionized Water	-----	0.20
Reagent E (Enzyme Solution)	0.20	-----

Run the reaction for 10 minutes. Maintain the pH of the reaction mix at pH 8.0 by the addition of small volumes of Reagent C. Record the volume of Reagent C used to maintain the pH.

CALCULATION:

$$\begin{aligned} [\text{NaOH}] &= \text{ml NaOH used Test} - \text{ml NaOH used Blank} \\ &\quad (\text{Molarity of NaOH}) [\text{NaOH}] (1000) \\ \text{Units/mg enzyme} &= \frac{\quad}{(10) (\text{mg enzyme/RM})} \end{aligned}$$

1000 = conversion from millimoles to micomoles (Unit definition)

10 = Time of assay (Unit Definition)

RM = Reaction Mix (initial volume = 10.2 ml)

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of L-a-phosphatidylcholine to L-a-lysophosphatidylcholine and a fatty acid per minute at pH 8.0 at 37°C.

INITIAL ASSAY CONCENTRATIONS:

In a 10.2 ml reaction mix, the initial concentrations are 2% L-a-phosphatidylcholine, 147 mM sodium chloride, 4.9 mM calcium chloride and 0.5 units phospholipase A2.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.