

Enzymatic Assay of PHOSPHOLIPASE A₂
(EC 3.1.1.4)

PRINCIPLE:

L-a-Lecithin + H₂O $\xrightarrow{\text{Phospholipase A}_2}$ L-a-Lysolecithin + Fatty Acid

Abbreviations used:

L-a-Lecithin = L-a-Phosphatidylcholine

L-a-Lysolecithin = L-a-Lysophosphatidylcholine

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, Sigma Prod. No. S-9625.)
- B. 100 mM Calcium Chloride Solution
(Prepare 100 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- C. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure¹.)
- D. 2.0% (w/v) Phosphatidylcholine Emulsion (Lecithin)
(Prepare by dissolving 4 grams of L-a-Phosphatidylcholine, Sigma Prod. No. P-5638, in a solution composed of 30 ml of Reagent A, 10 ml of Reagent B and 100 ml of deionized water. Stir 2-3 hours at 25°C to form an emulsion. Dilute to 200 ml with deionized water. Titrate with 1 M NaOH to pH 8.0 and continue to titrate to pH 8.0 (approximately 30 minutes) until the rate of decrease in pH is about 0.01 pH units/minute.)

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REAGENTS: (continued)

- E. Phospholipase A2 Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 units/ml of Phospholipase A2 in cold deionized water.)

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 if necessary. Then add:

Deionized Water	-----	0.20
Reagent E (Enzyme Solution)	0.20	-----

Run the reaction for approximately 10 minutes. Maintain the pH of the reaction mix at pH 8.0 by the addition of small volumes (0.05 ml) of Reagent C.² Record the volume of Reagent C used to maintain the pH at 8.0 and the time required.

CALCULATION:

[NaOH] = ml NaOH used for Test - ml NaOH used for Blank

$$\text{Units/mg enzyme} = \frac{(\text{Molarity of NaOH}) [\text{NaOH}] (1000)}{(T) (\text{mg enzyme/RM})}$$

1000 = conversion from millimoles to micromoles (Unit definition)

T = Time required to maintain the pH at 8.0 (in minutes)

RM = Reaction Mix

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.

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INITIAL ASSAY CONCENTRATIONS:

In a 10.2 ml reaction mix, the initial concentrations are 2.0% L-a-phosphatidylcholine, 147 mM sodium chloride, 4.9 mM calcium chloride and 0.5 units phospholipase A₂.

REFERENCES:

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

NOTES:

1. Standardization of NaOH solution is described in the cited reference.
2. The overall volume of NaOH addition (enzyme minus blank) should not exceed 0.5 ml (0.05 x 10) in order to obtain linear results.
3. 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.