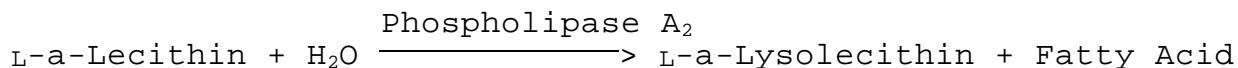


Enzymatic Assay of PHOSPHOLIPASE A₂¹
(EC 3.1.1.4)

PRINCIPLE:



Abbreviations used:

L-a-Lecithin = L-Phosphatidylcholine

L-a-Lysolecithin = L-a-Lysophosphatidylcholine

CONDITIONS: T = 25°C, pH = 8.9

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, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure².)
- D. 2.0% (w/v) Phosphatidylcholine Emulsion (Lecithin)
(Prepare by dissolving 4 grams of L-a-Phosphatidylcholine, Sigma Prod. No. P-3644, or L-a-Phosphatidylcholine, Sigma Prod. No. P-5638, in a solution consisting 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 8.9 and continue to titrate to pH 8.9 until the rate of decrease in pH is about 0.01 pH units/minute.)

Enzymatic Assay of PHOSPHOLIPASE A₂¹
(EC 3.1.1.1)

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 8.9 at 25°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 8.9 by the addition of
 small volumes (0.05 ml) of Reagent C.³ Record the volume
 of Reagent C used to maintain the pH at 8.9 and the time
 required.

CALCULATION:

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

$$\text{Units/ml enzyme} = \frac{(\text{Molarity of NaOH})[\text{NaOH}](1000)(\text{df})(10.2)}{(\text{T})(0.20)}$$

df = Dilution factor

1000 = Conversion factor from millimoles to micromoles
 (Unit definition)

T = Time required to maintain the pH at 8.9 (in minutes)

0.20 = Volume (in milliliter) of enzyme used

10.20 = Total volume (in milliliter) of assay

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

Enzymatic Assay of PHOSPHOLIPASE A₂¹
(EC 3.1.1.1)

CALCULATION: (continued)

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of L-a-phosphatidylcholine to L-a-lysophosphatidylcholine and a fatty acid per minute at pH 8.9 at 25°C.

INITIAL ASSAY CONCENTRATIONS:

In a 10.2 ml reaction mix, the initial concentrations are 2.0% (w/v) L-a-phosphatidylcholine, 147 mM sodium chloride, 4.9 mM calcium chloride and 0.5 unit phospholipase A₂.

REFERENCES:

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

NOTES:

1. This assay procedure is not to be used to assay Sigma Product Nos. P-1264, Phospholipase A2 from Bee Venom, Insoluble, P-6534, Phospholipase A2 from Porcine Pancreas, P-8913, Phospholipase A2 from Bovine Pancreas, and P-8685, Phospholipase from Streptomyces Violaceoruber.
2. Standardization of NaOH solution is described in the cited reference.
3. 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.
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.