Enzymatic Assay of PHOSPHOLIPASE A\textsubscript{2}  
(EC 3.1.1.4)

**PRINCIPLE:**

\[ \text{L-a-Lecithin} + \text{H}_2\text{O} \xrightarrow{\text{Phospholipase A}_2} \text{L-a-Lysolecithin} + \text{Fatty Acid} \]

Abbreviations used:

- L-a-Lecithin = L-a-Phosphatidylcholine
- L-a-Lysolecithin = L-a-Lysophosphatidylcholine

**CONDITIONS:**  \( T = 37 \, ^\circ\text{C}, \, \text{pH} = 8.0 \)

**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 Procedure\textsuperscript{1}.)

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\textdegree\text{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 A\textsubscript{2} Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 units/ml of Phospholipase A\textsubscript{2} 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Lecithin)</td>
<td>10.00</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Adjust to pH 8.0 at 37°C if necessary. Then add:

<p>| | | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.20</td>
<td>------</td>
</tr>
</tbody>
</table>

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.\textsuperscript{2} Record the volume of Reagent C used to maintain the pH at 8.0 and the time required.

CALCULATION:

\[ [\text{NaOH}] = \text{ml NaOH used for Test} - \text{ml NaOH used for Blank} \]

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

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:


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.