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

**PRINCIPLE:**

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

Abbreviations Used:
- L-a-Lecithin = L-a-Phosphatidylcholine
- L-a-Lysolecithin = L-a-Lysophosphatidylcholine

**CONDITIONS:**  
T = 37°C, pH 8.5, A\textsubscript{570nm}, Light path = 1 cm

**METHOD:**  
Colorimetric

**REAGENTS:**

A. 500 mM Tris HCl Buffer with 10 mM Calcium Chloride, pH 8.5 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503 and Calcium Chloride, Dihydrate, Prod. No. C-3881. Adjust to pH 8.5 at 37°C with 1 M HCl.)

B. 10 mM Calcium Chloride Solution (CaCl\textsubscript{2})  
(Prepare 50 ml in deionized water using Calcium Chloride, Dihydrate, Prod. No. C-3881.)

C. 2% (w/v) L-a-Phosphatidylcholine (L-a-Lecithin)  
(Prepare 10 ml in Reagent B using L-a-Phosphatidylcholine, Prod. No. P-5388. This solution is used for both the Substrate and Standard.)

D. 1.5% (w/v) Deoxycholate Solution  
(Prepare 10 ml in deionized water using Deoxycholic Acid, Sodium Salt, Prod. No. D-6750.)

E. 95% Ethanol (Nondenatured)

F. 25% (w/v) Ether  
(Prepare 20 ml in Reagent E using peroxidase-free Ether.)

Revised: 03/23/94  Page 1 of 4
Enzymatic Assay of PHOSPHOLIPASE A₂
(EC 3.1.1.4)

REAGENTS: (continued)

G. 2 M Hydroxylamine Solution
(Prepare 10 ml in deionized water using Hydroxylamine, Hydrochloride, Prod. No. H-9876.)

H. 14% (w/v) Sodium Hydroxide Solution (NaOH)
(Prepare 10 ml in deionized water using Sodium Hydroxide, Prod. No. S-0899.)

I. 3 N Hydrochloric Acid Solution (HCl)
(Prepare 10 ml in deionized water using Hydrochloric Acid, Prod. No. H-7020.)

J. 10% (w/v) Ferric Chloride solution (FeCl₃)
(Prepare 10 ml in deionized water using Ferric Chloride, Hexahydrate, Prod. No. F-2877.)

K. Phospholipase A₂ Enzyme Solution
(Immediately before use, prepare a solution containing 0.01 mg/ml of Phospholipase A₂ in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into 4 dram vials:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Reagent C (L-a-Lecithin)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Reagent D (Deoxycholate Solution)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

| Reagent K (Enzyme Solution) | 0.05   | ------ |
| Reagent B (CaCl₂)           | ------ | 0.05   |

Immediately mix by inversion and incubate at 37°C for 5 minutes at one minute intervals and remove 0.20 ml aliquots.

| Reagent F (Ether)       | 1.50   | 1.50   |
| Reagent G (Hydroxylamine Solution) | 0.20   | 0.20   |
| Reagent H (NaOH)        | 0.20   | 0.20   |
Enzymatic Assay of PHOSPHOLIPASE A$_2$
(EC 3.1.1.4)

PROCEDURE:  (continued)

Incubate at 25°C for 20 minutes. Then add:

\[
\begin{array}{lc}
\text{Test} & \text{Blank} \\
\text{Reagent I (HCl)} & 0.30 & 0.30 \\
\text{Reagent J (FeCl}_3\text{)} & 0.30 & 0.30 \\
\end{array}
\]

Mix by swirling and transfer to suitable cuvettes and record the $A_{570nm}$ for the Test and Blank.

COLORIMETRIC ASSAY:

Standard Curve:

(Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers.

\[
\begin{array}{cccccc}
\text{Std 1} & \text{Std 2} & \text{Std 3} & \text{Std 4} & \text{Blank} \\
\text{Reagent A (Buffer)} & 1.38 & 1.37 & 1.35 & 1.33 & --- \\
\text{Reagent C (L-a-Lecithin)} & 0.02 & 0.03 & 0.05 & 0.07 & --- \\
\text{Reagent D (Deoxycholate Soln)} & 0.70 & 0.70 & 0.70 & 0.70 & 0.70 \\
\text{Reagent B (CaCl}_2\text{)} & 0.05 & 0.05 & 0.05 & 0.05 & 0.05 \\
\end{array}
\]

Mix and incubate at 37°C for 5 minutes. Then pipette the following into suitable cuvettes.

\[
\begin{array}{cccccc}
\text{Standard Mixture} & 0.20 & 0.20 & 0.20 & 0.20 & 0.20 \\
\text{Reagent F (Ether)} & 1.50 & 1.50 & 1.50 & 1.50 & 1.50 \\
\text{Reagent G (Hydroxylamine)} & 0.20 & 0.20 & 0.20 & 0.20 & 0.20 \\
\text{Reagent H (NaOH Soln)} & 0.20 & 0.20 & 0.20 & 0.20 & 0.20 \\
\end{array}
\]

Incubate at 25°C for 20 minutes. Then add:

\[
\begin{array}{cccccc}
\text{Reagent I (HCl)} & 0.30 & 0.30 & 0.30 & 0.30 & 0.30 \\
\text{Reagent J (FeCl}_3\text{)} & 0.30 & 0.30 & 0.30 & 0.30 & 0.30 \\
\end{array}
\]

Mix by swirling and record the $A_{570nm}$ for both the Standards and Blank using a suitable spectrophotometer.

CALCULATIONS:

\[\Delta A_{570nm \text{ Standard}} = A_{570nm \text{ Standard}} - A_{570nm \text{ Standard Blank}}\]

Prepare a standard curve by plotting the $A_{570nm}$ of the Standard vs the micromoles of L-a-Phosphatidylcholine. Use the slope (M) to determine the micromoles of L-a-Phosphatidylcholine of the Test mixture.
Enzymatic Assay of PHOSPHOLIPASE A$_2$
(EC 3.1.1.4)

CALCULATIONS: (continued)

\[
\Delta A_{570\text{nm}} = \frac{A_{570\text{nm}} \text{ Test} - A_{570\text{nm}} \text{ Blank}}{\text{Time in minutes}}
\]

\[
\text{units/mg enzyme} = \frac{\Delta A_{570\text{nm}/\text{min}}}{(M) \ (0.5) \ (\text{mg/enzyme/ml RM}) \ (0.8)}
\]

M = Slope of Std Curve
RM = Reaction Mix
0.5 = Conversion factor since the lysolecithin which is formed has one half the absorbance of lecithin at 570 nm.
0.8 = Conversion factor since the MW is assumed to be 800 to obtain micromolar units from mg units.

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of L-$\alpha$-phosphatidylcholine to L-$\alpha$-lysophosphatidylcholine and a fatty acid per minute at pH 8.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.15 ml reaction mix, the final concentrations are 163 mM Tris, 0.07% (w/v) L-$\alpha$-phosphatidylcholine, 0.49% (w/v) deoxycholate and 0.0005 mg phospholipase A$_2$.

NOTES:

1. All products 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.