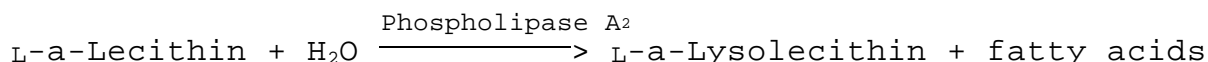


Enzymatic Assay of PHOSPHOLIPASE A₂
(EC 3.1.1.4)

PRINCIPLE:



Abbreviations Used:

L-a-Lecithin = L-a-Phosphatidylcholine

L-a-Lysolecithin = L-a-Lysophosphatidylcholine

CONDITIONS: T = 37°C, pH 8.5, A_{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 (CaCl₂)
(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.)

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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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.70	0.70
Reagent C (L-a-Lecithin)	0.70	0.70
Reagent D (Deoxycholate Solution)	0.70	0.70

Equilibrate to 37°C. Then add:

Reagent K (Enzyme Solution)	0.05	-----
Reagent B (CaCl ₂)	-----	0.05

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

Test Mixture	0.20	-----
Blank Mixture	-----	0.20
Reagent F (Ether)	1.50	1.50
Reagent G (Hydroxylamine Solution)	0.20	0.20
Reagent H (NaOH)	0.20	0.20

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PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent I (HCl)	0.30	0.30
Reagent J (FeCl ₃)	0.30	0.30

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.

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Blank</u>
Reagent A (Buffer)	1.38	1.37	1.35	1.33	---
Reagent C (L-a-Lecithin)	0.02	0.03	0.05	0.07	---
Reagent D (Deoxycholate Soln)	0.70	0.70	0.70	0.70	0.70
Reagent B (CaCl ₂)	0.05	0.05	0.05	0.05	0.05

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

Standard Mixture	0.20	0.20	0.20	0.20	0.20
Reagent F (Ether)	1.50	1.50	1.50	1.50	1.50
Reagent G (Hydroxylamine)	0.20	0.20	0.20	0.20	0.20
Reagent H (NaOH Soln)	0.20	0.20	0.20	0.20	0.20

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

Reagent I (HCl)	0.30	0.30	0.30	0.30	0.30
Reagent J (FeCl ₃)	0.30	0.30	0.30	0.30	0.30

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.

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CALCULATIONS: (continued)

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

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

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-a-phosphatidylcholine to L-a-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-a-phosphatidylcholine, 0.49% (w/v) deoxycholate and 0.0005 mg phospholipase A₂.

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.