

Phospholipase A2 (PLA2)

Cat. No.: P1523405 | Pack size: 100 U / 500 U / 2.5 KU | Storage: 2–8 °C, protected from light, desiccated

Overview

Phospholipase A2 (PLA2) is a calcium-dependent, heat-stable enzyme that catalyzes the hydrolysis of glycerophospholipids at the sn-2 position of cellular membranes, thereby releasing Arachidonic Acid (AA). Phospholipase A2 is a key mediator in the biosynthesis of pro-inflammatory lipid mediators, critically involved in inflammatory processes. Phospholipase A2 can be used for cardiovascular and inflammatory diseases research.

Preparation and Specification

Appearance: Colorless to brownish solution.

Specific activity: More than 200 U/mL.

Properties

Property	Specification / Condition
Substrate specificity	See Table 1
Molecular weight	15 kDa (Sephadex G-100)
Isoelectric point	pH 7.51
Michaelis constants	Lecithin 5.0×10^{-3} M
Optimum pH	7.3–8.3 (Figure 1)
pH stability	6.0–9.5 (55 °C, 10 min) (Figure 2)
Thermal stability	Stable at 50 °C and below (pH 8.0, 10 min, + CaCl ₂) (Figure 3)
Stabilizer	Ca ²⁺
Activators	Ca ²⁺ , Non-ionic detergents
Inhibitor	EDTA

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of lecithin when coupled with lysophospholipase, glycerophosphorylcholine phosphodiesterase and choline oxidase.

Table 1. Substrate specificity

Substrate	Relative activity (%)
Phosphatidylcholine	100
Phosphatidylethanolamine	30
Phosphatidic Acid	10
Lysophosphatidylcholine	0
Triolein	0
Trilaurin	0

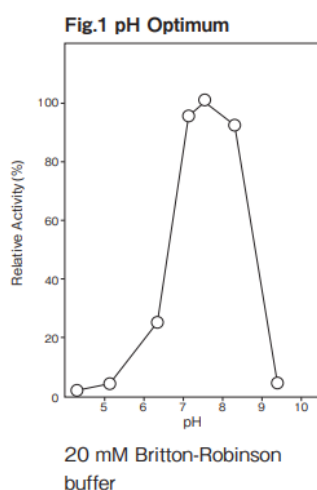


Figure 1. pH Optimum

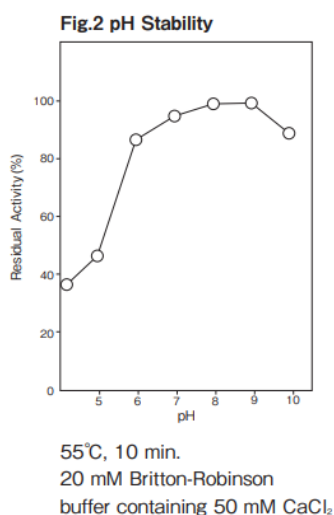


Figure 2. pH Stability

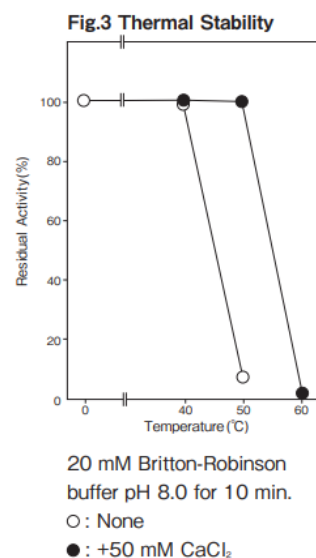


Figure 3. Thermal Stability

Assay

Principle

The assay is based on the increase in absorbance at 500 nm as the formation of quinoneimine dye proceeds in the following reactions:

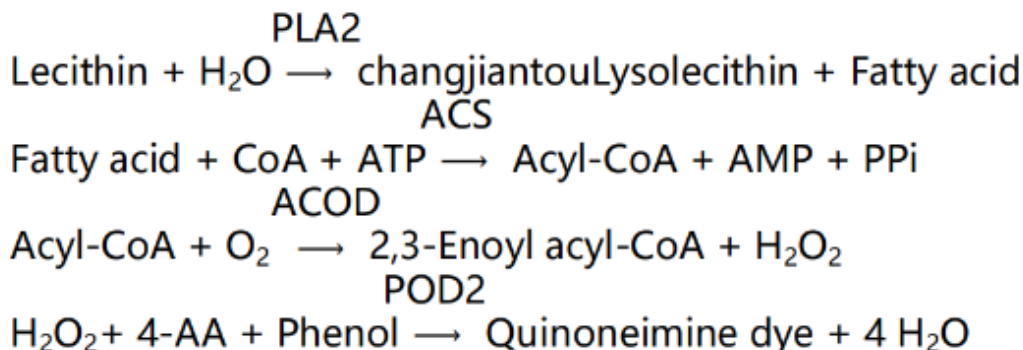


Figure 4. Principle of the phospholipase A2 activity assay

ACS: Acyl-CoA synthetase.

ACOD: Acyl-CoA oxidase.

ATP: Adenosine triphosphate.

Unit definition

One unit is defined as the amount of enzyme which liberates 1 μmole of fatty acid per minute at 37 °C under the conditions specified in the assay procedure.

Reagents

1. Reaction mixture for the first reaction

0.2 M Tris-HCl buffer, pH 8.0 0.20 mL

20 mM DPPC solution^a 0.05 mL

1 M CaCl₂ solution 0.025 mL

0.2 M ATP solution, pH 7.0 0.01 mL

0.1 M CoA solution, pH 6.0^b 0.02 mL

10.6 U/mL ACS solution^c 0.05 mL

Distilled water 0.145 mL

a: 20 mM DPPC solution — Dissolve 147 mg of DPPC with 10 mL of 2% (W/V) Triton X-100 solution.

b: 0.1 M CoA solution pH 6.0 — Dissolve 7.85 g (purity calculation) of CoA with 80 mL of distilled water and adjust pH to 6.0 (25 °C) with 4 N NaOH and add distilled water to make a total of 100 mL.

c: 10.6 U/mL ACS solution — Dissolve 106 U of ACS with 10 mL of Tris-HCl buffer pH 8.0.

DDPC: Dipalmytoylphosphatidylcholine.

Procedure

1. Pipette accurately 0.50 mL of reaction mixture for the first reaction into a small test tube and preincubate at 37 °C.

2. After 5 min, add exactly 50 μL of enzyme sample and mix to start the first reaction at 37 °C.

※ In the case of a test blank, add 50 μL of enzyme dilution buffer in place of enzyme solution at this point.

3. After 10 min, add 0.5 mL of 20 mM NEM and add 0.5 mL of reaction mixture for the second reaction after 15 seconds later and mix to start the second reaction at 37 °C.
4. At 5 min after starting the reaction, add 1.5 mL of the reaction stopper to stop the reaction.
5. Measure the absorbance at 500 nm.

$$\Delta A = (A_s - A_b) \leq 0.20 \text{ Abs}$$

Absorbance sample : A_s

blank : A_b

Calculation

$$\text{Activity (U/mg)} = \{(\Delta A/10)/(12.0 \times 1/2)\} \times 3.05/0.05 \times D$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm ($\text{cm}^2/\mu\text{mole}$).

1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye.

10 : reaction time (min).

3.05 : final volume (mL).

0.05 : volume of enzyme solution (mL).

D : times of dilution in enzyme solution.

Specifications

Item	Specification
Product Name	Phospholipase A2 (PLA2), CAS No.9001-84-7
Synonyms	Phosphatidylcholine 2-acylhydrolase phospholipase A2 lecithinase A phosphatidase phosphatidolipase phosphatidylcholine 2-acylhydrolase PLA2G1B PLA2 sPLA(2)-IB
Grade	ActiBioPure™, Bioactive, High Performance, Recombinant, EnzymoPure™
Specifications & Purity	Bioactive, Recombinant, ActiBioPure™, High Performance, EnzymoPure™, ≥200 U/mL; Protein concentration: See COA
Bioactivity	≥200 U/mL
CAS	9001-84-7
Enzyme Commission Number	EC 3.1.1.4
Molecule Type	Enzyme

Storage and Shipping

Item	Information
Concentration	≥200 U/mL; Protein concentration: See COA
Storage	Store at 2–8 °C, Protected from light, Desiccated
Shipped In	Wet ice
Stability And Storage	Store at 2–8 °C long term (12 months). Upon receipt, it is recommended to aliquot. Store in the dark. Desiccated.
Unit definition	One unit is defined as the amount of enzyme which liberates 1 μmole of fatty acid per minute at 37 °C under the conditions specified in the assay procedure.

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