CREATIVE BIOMART®
Assay Kit

cPLA2 Assay Kit

Product Information

Cat.No.

Kit-0696

Product Overview

Phospholipase A2 catalyzes the hydrolysis of fatty acids at the sn-2 position of glycerophospholipids, yielding a free fatty acid and a lysophospholipid as products. The release of arachidonic acid from membrane phospholipids by these enzymes is believed to be the key step in the biosynthesis of eicosanoids. There are primarily three different kinds of phospholipase A2. They are secretory (sPLA2), calcium-dependent cytosolic (cPLA2), and calcium-independent cytosolic (iPLA2) phospholipase A2. Of these three different types of enzymes, only the cPLA2 exhibits specificity towards arachidonic acid whereas all others can hydrolyze any fatty acid at the sn-2 position. Arachidonoyl Thio-PC is a substrate for cPLA2 by virtue of the presence of arachidonic acid at the sn-2 position of the glycerophospholipid. Hydrolysis of the arachidonoyl thioester bond at the sn-2 position by PLA2 releases a free thiol which can be detected by DTNB (5,5''-dithiobis (2-nitrobenzoic acid)). This assay can be used to determine the activity of cPLA2 in purified preparations, cell cultures, or tissue homogenates that are known to contain only cPLA2. Use of this assay with preparations containing more than one type of PLA2 will result in the measurement of total PLA2 activity rather than cPLA2 alone. Isozyme-specific cPLA2 activity can be measured by excluding sPLA2 or inhibiting iPLA2 activities in the assay. Each kit contains cPLA2 assay buffer, DTNB/EGTA, Arachidonoyl Thio-PC (substrate), bee venom PLA2 (control), bromoenol lactone solution, a 96 well plate, and complete instructions.

Description

Phospholipases A2 (PLA2s) catalyze the hydrolysis of fatty acids at the sn-2 position of glycerophospholipids, yielding a free fatty acid and a lysophospholipid as products.1 The release of arachidonic acid from membrane phospholipids by these enzymes is believed to be the key step in the biosynthesis of eicosanoids.2 There are primarily three different kinds of PLA2s, they are secretory (sPLA2), calcium-dependent cytosolic (cPLA2), and calciumindependent (iPLA2) PLA2s. Of these three different types of enzymes, only the cPLA2 exhibits specificity towards arachidonic acid

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whereas all others can hydrolyze any fatty acid at the sn-2 position. Arachidonoyl thio-PC is a synthetic substrate that can be used to detect phospholipase activity.3 Hydrolysis of the arachidonoyl thioester bond at the sn-2 position by PLA2 releases a free thiol which is detected by DTNB (5,5''-dithio-bis (2-nitrobenzoic acid); Ellman''s reagent). This assay can be used to determine the activity of cPLA2 in purified preparations, cell cultures, or tissue homogenates. Use of this assay with preparations containing more than one type of PLA2 will result in the measurement of total PLA2 activity rather than cPLA2 alone unless specific inhibitors or purification procedures are used.

Usage

Please read these instructions carefully before beginning this assay.For research use only. Not for human or diagnostic use.

Storage

Stability: 1 year;Storage: -20°C;This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Kit Components

cPLA2 Assay Buffer: 1 vial;DTNB/EGTA: 4 vials;Arachidonoyl Thio-PC (Substrate): 2 vials;Bee venom PLA2 Control: 1 vial;Bromoenol Lactone Solution: 1 vial;96-Well Solid Plate (Colorimetric Assay): 1 plate;96-Well Cover Sheet: 1 cover

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