CREATIVE BIOMART®
Assay Kit

Phospholipase A2 Activity Assay Kit (Fluorometric)

Product Information

Cat.No. Kit-1082

Product Overview

Phospholipase A2 Activity Assay Kit provides a quick, sensitive and simple way for measuring PLA2 activity in various biological samples. In this assay, active PLA2 cleaves a synthetic thiophospholipid, producing a lysothiophospholipid which reacts with a fluorogenic probe to produce a highly fluorescent product detectable in the visible range (Ex/Em= 388/513 nm). The assay is simple to perform, high-throughput adaptable and can detect less than 0.1 mU of PLA2 activity.

Applications

Measurement of phospholipase A2 activity in various tissues/cells, venoms, secretory fluids.

Storage

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

Size

100 assays

Kit Components

PLA2 Assay Buffer 25 ml
PLA2 Substrate 40 µl
PLA2 Probe 100 µl
Fluorescence Standard 1 vial
Bee Venom Positive Control 1 vial

Materials Required but Not Supplied

Black 96-well plate with flat bottom
Multi-well spectrophotometer (ELISA reader)
30 kDa Spin Columns

Compatible Sample Types

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Venoms and secretory fluids (snake venom, bee venom, synovial fluid, etc.)
Soft tissue homogenates (liver, intestine, etc.)
Cultured cells (adherent or suspension cells)

Preparation

Reagent Preparation:
PLA2 Assay Buffer: Warm to room temperature before use. Store at 4°C or -20°C.
PLA2 Substrate: Allow to thaw to room temperature before use. Aliquot as desired and store at -20°C. While using, keep on ice after dilution. Avoid repeated freeze/thaw cycles. Use within two months.
PLA2 Probe: Allow to thaw to room temperature before use. Aliquot and store at -20°C.
Fluorescence Standard: Reconstitute with 110 µl DMSO to generate 1 mM Standard solution. Aliquot and store at -20°C. Allow to thaw to room temperature before use and keep at room temperature while in use. Use within two months.
Bee Venom Positive Control: Reconstitute with 22 µl PLA2 Assay Buffer and mix thoroughly. Aliquot and store at -80°C. Use within two months. Keep on ice while in use.

Assay Protocol

1. Sample Preparation:
a. Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice-cold PLA2 Assay Buffer and keep on ice for 10 min. Centrifuge at 10,000 x g for 10-15 min at 4°C and transfer the supernatant to a fresh, prechilled microfuge tube. Add 5-25 µl sample per well in a black 96 well plate and adjust the volume to 50 µl with PLA2 Assay Buffer. For positive control: dilute the required amount of Bee Venom Positive Control 100 times with PLA2 Assay Buffer. Add 10 µl of the diluted Bee Venom Positive Control per well into the desired well(s) and adjust the final volume to 50 µl with PLA2 Assay Buffer. Use the diluted positive control solution within one hour of preparation.
Notes: Complex biological samples such as tissue homogenates and cell lysates may contain a mixture of PLA2 subtypes; detected sample activity, therefore reflects total PLA2 activity. The activity of low molecular weight (secretory) Phospholipase A2 enzymes (sPLA2s) may be determined by ultrafiltration of the sample using 30 kDa MWCO spin columns to remove any high molecular weight (cytosolic) phospholipase A2 enzymes (cPLA2s and iPLA2s). Add sample to the spin column,

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centrifuge at 10,000 x g at 4°C for 10 min and collect the filtrate, which will contain only sPLA2s.
For unknown samples, we suggest doing a pilot experiment to ensure readings are within the range of the standard curve. Samples with extremely high PLA2 activity (such as venoms and other secretory fluids) may be diluted with PLA2 Assay Buffer.
We recommend using a protease inhibitor cocktail to prevent enzyme degradation and measuring sample protein concentration using the Bradford reagent.
For samples exhibiting significant background, prepare parallel sample well(s) as background controls.
2. Fluorescence Standard Curve:
Dilute the Fluorescence Standard stock solution 10 times with dH2O just prior to use to obtain a 0.1 mM working solution. Add 0, 2, 4, 6, 8 and 10 µl of the 0.1 mM Fluorescence Standard into a series of wells in a 96 well plate to generate 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol/well of Fluorescence Standard. Adjust the volume to 100 µl/well with PLA2 Assay Buffer.
3. Fluorogenic Probe Preparation:
Dilute the PLA2 Probe stock solution 10 times with dH2O to obtain a 1X working solution. Add 10 µl of the 1X working solution to all the wells except those containing the standards.
4. Substrate Preparation and Reaction:
Dilute the PLA2 Substrate stock solution 100 times with PLA2 Assay Buffer to obtain a working solution. Make enough to add 40 µl per well (for example, for 10 reactions, add 4 µl of PLA2 Substrate stock to 396 µl PLA2 Assay Buffer to make 400 µl of working solution). Vortex and add 40 µl to each test and positive control well. Do not add PLA2 Substrate solution to the background control wells.
Test Sample/Positive Control Sample Background Control
Substrate Solution (1X) 40 µl —
PLA2 Assay Buffer — 40 µl
Note: The PLA2 probe solution (1X) and the Substrate solution (1X) should be added to the wells sequentially as per protocol and not premixed as a single solution.
5. Measurement:
Immediately begin measuring the fluorescence at Ex/Em= 388/513 nm in kinetic mode for 45-60 mins at 37°C. Measurement time for the linear phase of the reaction depends on the PLA2 activity in samples. We recommend measuring the sample fluorescence in kinetic mode to ensure that the

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measurements recorded are within the linear range of the reaction.
Note: The Fluorescence Standard Curve may be read separately in endpoint mode.

Analysis

Subtract the 0 nmol Fluorescence Standard reading from all standard curve readings, plot the standard curve and calculate the slope. For each reaction well (including sample background and positive control wells), choose two time points (t1 and t2) in the linear phase, obtain the corresponding fluorescence values at those points (RFU1 and RFU2) and determine the change in fluorescence over the time interval: ΔF = RFU2 – RFU1. If sample background control reading is significant, subtract the background control reading from its paired sample reading. PLA2 metabolic activity is obtained by applying the background-corrected ΔF values to the Fluorescence Standard curve to get B nmol of PLA2 Substrate metabolized during the reaction time ($\Delta T = T2 - T1$).
Sample Phospholipase A2 Activity = $B/(\Delta T \times V) \times D$ = nmol/min/ml = mU/ml
Where: B = metabolite amount from standard curve (nmol).
Δt = reaction time of linear phase (min).
V = sample volume added into the reaction well (ml).
D = Sample dilution factor (if applicable)
Unit Definition: One unit of Phospholipase A2 is the amount of enzyme that generates 1.0 µmole of lysothiophospholipid metabolite per min at pH 7.5 at 37°C.
Note: Activity of the high molecular weight Phospholipase A2 enzymes (cPLA2 and iPLA2 type enzymes) can be calculated by subtracting the activity of the secretory Phospholipase A2 enzymes (filtered fraction) from the total Phospholipase A2 activity.