CREATIVE BIOMART®
Assay Kit

5-Lipoxygenase Inhibitor Screening Kit (Fluorometric)

Product Information

Cat.No. Kit-2318

Product Overview

5-Lipoxygenase (5-LOX) is a non-heme iron-containing dioxygenase that converts unsaturated fatty acids to epoxides; for example the synthesis of leukotrienes from arachidonic acid. It is involved in processes like cell proliferation, differentiation and inflammation and has been implicated in inflammation and hyper proliferation mediated diseases like asthma, rheumatoid arthritis and cancer. Research for identifying potential remedies for such diseases involves the identification of inhibitors of 5-lipoxygenase. 5-Lipoxygenase Inhibitor Screening Kit uses Zileuton, a well-known 5-lipoxygenase inhibitor as positive control, and provides a simple and fast method to screen/ characterize potential 5-lipoxygenase inhibitors.

Storage

Upon arrival, store the kit at -80°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay. Components are stable for at least three months.

- LOX Assay Buffer: Warm to room temperature before use.
- LOX Probe and Zileuton: Aliquot and store at -80°C in the dark.
- LOX Substrate: Store at -80°C.

5-LOX Enzyme: Aliquot and store at -80°C. Avoid repeated freeze thaw cycles. Note: Keep all components on ice while performing the assay.

Size

100 assays

Kit Components

LOX Assay Buffer 25 ml
LOX Probe 200 µl
LOX Substrate 6 µl
Zileuton 100 µl
5-LOX Enzyme 300 U

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Materials Required but Not Supplied

96-well white plate with flat bottom
Multi-well spectrophotometer (ELISA reader)
Deionized water
DMSO (anhydrous)

Assay Protocol

1. Test Compound preparation: Dissolve the test compound in appropriate solvent. Prepare at such concentration so volume of test compound solution added to a well is no more than 2 µl in the final 100 µl reaction volume per well. Add 2µl test compound to each well of the 96 well white plate. For "Solvent Control", add 2µl of the solvent used to prepare test compound solution at its final concentration in test wells, and for "Inhibitor Control" add 2µl of Zileuton, the provided LOX inhibitor. Bring up the volume to 40 µl in each well by adding 38 µl of LOX Assay Buffer. For the "Enzyme Control" well, add 40 µl LOX Assay Buffer to a well.
2. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Mix containing:
LOX Assay Buffer 36 µl
LOX Probe 2 µl
5-LOX Enzyme 2 µl
Mix well and add Reaction Mix to wells containing the Enzyme Control, Inhibitor Control, "Solvent Control" and Test Compounds. Incubate plate at RT for 10 minutes before adding substrate. There should be no bubbles in the wells.
3. LOX Substrate: Dilute the provided LOX substrate (12500 X) in LOX Assay Buffer using 1:25 dilution factor to obtain a 500 X solution. Depending on the number of reactions to be performed, dilute the 500 X solution in LOX Assay Buffer at 1:100 to get 5X solution. 20 µl of 5X solution will be needed per reaction. Make up enough substrate depending on the number of reactions. Final substrate working solution should be kept on ice and be used up within the same day. Immediately store the remaining stock solution at -20°C. Add 20 µl of 5X LOX Substrate to each well using a multichannel pipette.
Note: have the plate reader ready at Ex/Em 500/536 nm on kinetic mode set to record fluorescence

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every 30 seconds.
4. Measurement: Start recording fluorescence at Ex/Em 500/536 nm on the second minute after the addition of the substrate at 30 second intervals for 10 - 20 minutes.
5. Calculation: Obtain Δ RFU for all samples by subtraction RFU at time t1 form RFU at time t2, such that t2 and t1 is within a linear range of the assay. Calculate slope for all samples, including "enzyme control" by dividing ΔRFU by time Δt (t2 – t1). If "Solvent Control" slope is different from "Enzyme Control" slope, use its values instead of "Enzyme Control" in the calculations shown below.
% Inhibition = [slope of (enzyme control) - slope of (test compound)] / slope of (enzyme control) *100
% Relative activity = slope of (test compound) / slope of (enzyme control) *100

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