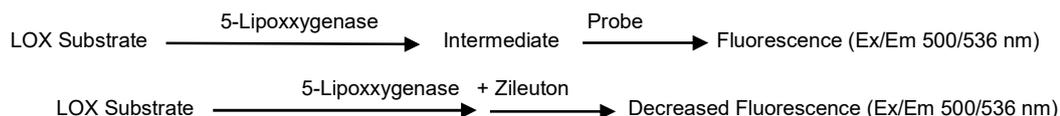


5-Lipoxygenase Inhibitor Screening Kit (Fluorometric) (#BN01138)

(Catalog # BN01138; 100 assays; Store at -80°C)

I. Introduction:

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. Assay Genie's 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.



II. Applications:

- Screening of potential inhibitors of 5-lipoxygenase

III. Kit Contents:

Components	BN01138	Cap Code	Part Number
LOX Assay Buffer	25 ml	WM	BN01138-1
LOX Probe	200 µl	Red	BN01138-2
LOX Substrate	6 µl	Green	BN01138-3
Zileuton	100 µl	Orange	BN01138-4
5-LOX Enzyme	300 U	Blue	BN01138-5

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

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.*

VI. 5-Lipoxygenase Inhibitor Screening 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:

	Reaction Mix
LOX Assay Buffer	34 µl
LOX Probe	2 µl
5-LOX Enzyme	4 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 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 30 - 60 minutes.

5. Calculation: Obtain Δ RFU for all samples by subtraction RFU at time t_1 from RFU at time t_2 , such that t_2 and t_1 is within a linear range of the assay. Calculate slope for all samples, including "enzyme control" by dividing Δ RFU by time Δt ($t_2 - t_1$). If "Solvent Control" slope is different from "Enzyme Control" slope, use its values instead of "Enzyme Control" in the calculations shown below.

$$\% \text{ Inhibition} = \frac{[\text{slope of (enzyme control)} - \text{slope of (test compound)}]}{\text{slope of (enzyme control)}} \times 100$$

$$\% \text{ Relative activity} = \frac{[\text{slope (test compound)}]}{\text{slope (enzyme control)}} \times 100$$

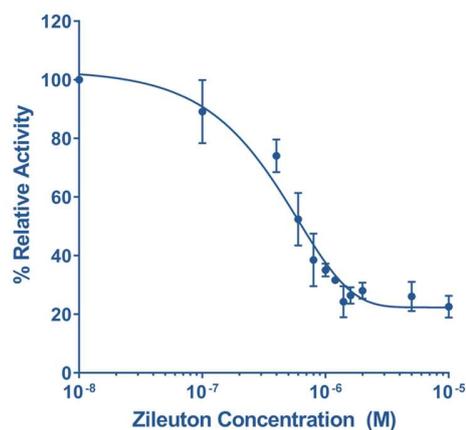


Figure: Inhibition of 5-Lipoxygenase enzyme activity by Zileuton. IC₅₀ of zileuton was determined to be 0.53 ± 0.04 μM. Assays were performed using kit protocol.

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