Amplite® Fluorimetric Lipoxygenase Inhibitor Screening Kit *Green Fluorescence*

Catalog number: 15252
Unit size: 100 tests

Component	Storage	Amount (Cat No. 15252)
Component A: LOX Green	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µl)
Component B: LOX Assay Buffer	Freeze (< -15 °C)	10 ml
Component C: LOX Enzyme	Freeze (< -15 °C)	1 vial (50 µg)
Component D: Arachidonic Acid	Freeze (< -15 °C)	1 vial (50 µl)
Component E: LOX Inhibitor	Freeze (< -15 °C)	1 vial (25mM, 20µl/vial)

OVERVIEW

The Amplite® Fluorimetric Lipoxygenase Inhibitor Screening Kit offers a robust, quick and convenient method for screening and evaluating compounds that inhibit lipoxygenase (LOX) activity in biological samples. Lipoxygenases are iron-containing oxidoreductase enzymes that catalyze the dioxygenation of unsaturated fatty acids, such as arachidonic acid and linoleic acid, to produce hydroperoxides. These hydroperoxides act as intermediates in the production of leukotrienes and lipoxins, which are signaling molecules involved in inflammatory responses, cancer, and other biological processes.

Our Amplite® Lipoxygenase Inhibitor Screening Kit utilizes a fluorescence detection system to monitor the inhibition of lipoxygenase activity. The substrate is oxidized by lipoxygenase in the sample, producing a fluorogenic compound that generates a fluorescence signal (Ex/Em = 490/530 nm). The presence of an inhibitor reduces the enzymatic activity, leading to a decrease in the fluorescence signal. This assay provides a simple, reliable solution for identifying potential inhibitors of lipoxygenase.

This kit is compatible with fluorescence microplate readers and is ideal for high-throughput screening of inhibitors, studying enzyme kinetics, or investigating disease-related pathways involving lipoxygenase.

AT A GLANCE

Protocol summary

1. Prepare LOX enzyme solution (40 µL)
2. Prepare inhibitor serial dilution and add 10 µL
3. Incubate enzyme with inhibitor for 5-10 minutes at RT
4. Add LOX working solution (50 µL)
5. Incubate 10-30 min at RT
6. Monitor fluorescence intensity at Ex/Em = 490 nm/ 530 nm (Cutoff= 515 nm)

Important notes
Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument: Fluorescence microplate reader
Excitation: 490 nm
Emission: 530 nm
Cutoff: 515 nm
Recommended plate: Solid black

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15252

LOX Inhibitor Standard Solution:
Add 8 µL of LOX Inhibitor (Component E) to 992 µL PBS to get 200 µM of LOX inhibitor (Std 7). Take 500 µL (Std 7) and perform 1:2 serial dilutions in PBS to get serially diluted standards (Std 6-Std 1).

PREPARATION OF WORKING SOLUTION

LOX Enzyme (Lipooxygenase Enzyme):

Add 50 µL PBS to LOX Enzyme (Component C) to prepare 1 mg/ mL of stock solution. Mix well by pipetting up and down. Store at < -15 °C. Avoid repeated freeze/thaw cycles.

Add 4 µL of 1 mg/ml LOX to 996 µL PBS+0.1% BSA (not included) to get 4 µg/ml LOX enzyme solution.

LOX Working Solution

Add 20 µL of Arachidonic Acid (Component D), 50 µL LOX Green (Component A) into 5 mL of LOX Assay Buffer (Component B), and mix well to make a LOX working solution.

Note: This LOX working solution should be prepared freshly before the experiment, and kept from light. 5 mL is for 100 tests; please prepare the amount of LOX working solution as needed proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1: Layout of LOX inhibitor standards and test samples in a solid black 96-well microplate. STD = LOX inhibitor Standards (STD 1-STD 7, 0.6 to 40 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
STD1	STD1	...	...
STD2	STD2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2. Reagent composition for each well.

Well	Volume (total 50 µL)
STD1-STD7	40 µL LOX enzyme dilution+10 µL LOX inhibitor serial dilution
BL Control	PBS+0.1% BSA: 50 µL
TS	LOX enzyme solution 40 µL+10 µL test inhibitor sample

Positive control	LOX enzyme solution 40 µL+10 µL PBS

1. Prepare LOX inhibitor standards (STD1-7), blank controls (BL), LOX positive control and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Incubate LOX enzyme solution with LOX inhibitor solution for 10 min at RT.
3. Add 50 µL of LOX Working Solution to each well of standard, blank control, positive control and test samples. For a 384-well plate, add 25 µL of LOX Working Solution into each well instead.
4. Incubate at 10–20 minutes at RT, protected from light.
5. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 490 nm/ 530 nm (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence reading in blank wells (with PBS+0.1%BSA only) is used as a control, and is subtracted from the values of those wells with the standards, positive control and test samples. The typical inhibition of LOX with LOX inhibitor was shown in Figure 1. To calculate the IC50 of the test inhibitor samples, we recommend using the Online Linear Regression Calculator which can be found at: https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

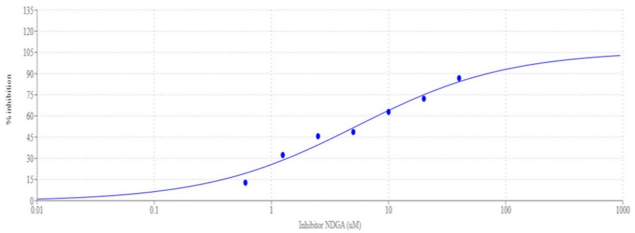

Figure 1. Inhibitor dose response was measured with Amplite® Fluorimetric LOX inhibitor screening kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em = 490 nm/530 nm (cutoff = 515 nm).

DISCLAIMER

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