Amplite® Fluorimetric Lipooxygenase Activity Assay kit *Green Fluorescence*

Catalog number: 15251
Unit size: 100 tests

Component	Storage	Amount (Cat No. 15251)
Component A: Lox Green	Freeze (< -15 °C), Minimize light exposure	1 vial (100µl)
Component B: LOX Assay Buffer	Freeze (< -15 °C)	10 ml
Component C: LOX Positive Control	Freeze (< -15 °C)	1 vial (50 µg)
Component D: Arachidonic Acid	Freeze (< -15 °C)	1 vial (50 µl)
Component E: Rhodamine 123 Standard	Freeze (< -15 °C)	1 vial (50µl)

OVERVIEW

The Amplite® Fluorimetric Lipooxygenase Activity Assay kit offers a robust, convenient and rapid method for measuring lipoxygenase (LOX) activity in biological samples. Lipoxygenases are iron-containing oxidoreductase enzymes that are involved in the dioxygenation of unsaturated fatty acids, such as arachidonic acid and linoleic acid, to produce hydroperoxides. These hydroperoxides serve as intermediates for the production of leukotrienes and lipoxins, which are signaling molecules that regulate inflammatory responses involved in inflammation, cancer, and other biological processes.

Our Amplite® Fluorimetric Lipooxygenase Activity Assay kit detects lipoxygenases based on a fluorescence detection system. The substrate is oxidized by lipoxygenase present in the sample, producing a fluorigenic compound resulting in an enhanced fluorescence signal with Ex/Em=490/530nm. The generated signal is directly proportional to the enzymatic activity, enabling accurate quantification of the enzyme. This kit provides a simple, reliable solution for detecting lipoxygenase activity in various biological samples, including cell lysates, serum, purified enzymes, and tissue homogenates. It is compatible with fluorescence microplate readers and is ideal for studying enzyme kinetics, screening inhibitors, or investigating disease-related pathways.

AT A GLANCE

Protocol summary

1. Prepare test samples along with LOX positive control and Rhodamine standard (50 µL)
2. Add LOX working solution (50 µL)
3. Incubate for10–30 minutes at RT
4. 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/15251

Rhodamine 123 Standard

Add 2 µL of Rhodamine 123 Standard (Component E) to 998 µL PBS+0.1% BSA (not included) to get 10 µM of Rhodamine 123 standard (Std7). Take 500 µL (Std7) and perform 1:2 serial dilutions in PBS+0.1% BSA to get serially diluted standards (Std 6-Std 1).

PREPARATION OF WORKING SOLUTION

Lipooxygenase Positive Control (LOX Positive Control)

Add 50 µL PBS to LOX Positive Control (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 enzyme to 996 µL PBS+0.1% BSA to get 4 µg/ml LOX enzyme. LOX working solution is not stable and should be used promptly.

LOX Working Solution

Add 20 µL of Arachidonic Acid (Component D) into 5.0 mL of LOX Assay Buffer (Component B), and then add 50 µL of LOX Green (Component A) and mix well to make LOX working solution.

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1: Layout of Rhodamine 123 standards and test samples in a solid black 96-well microplate. STD = Rhodamine123 Standards (STD 1-STD 7, 0.15 to 10 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	Reagent
STD1-STD7	50 µL	Serial Dilutions (0.15 to 10 µM)
BL Control	50 µL	PBS+0.1% BSA
LOX Positive Control	50 µL	LOX positive control 4 µg/ml
TS	50 µL	LOX Sample

1. Prepare Rhodamine123 standards (STD1-7), Blank Controls (BL), LOX Positive Control and LOX 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. Add 50 µL of LOX Working Solution to each well of standard, blank control, LOX positive control and test samples. For a 384-well plate, add 25 µL of LOX working solution into each well instead.
3. Immediately after addition of the LOX Working Solution, measure the fluorescence (RFU) using the preset plate reader settings (Ex = 490 nm/Em = 530 nm, Cutoff=515nm) in kinetic mode reading every 30 seconds for a total of 20 minutes at room temperature. The standard curve can be read together with the sample or at the end of the incubation time.

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the standards, positive control and test samples. The standard curve of Rhodamine 123 is shown in Figure 1. To calculate the LOX activity of the samples according to the standard curve, 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

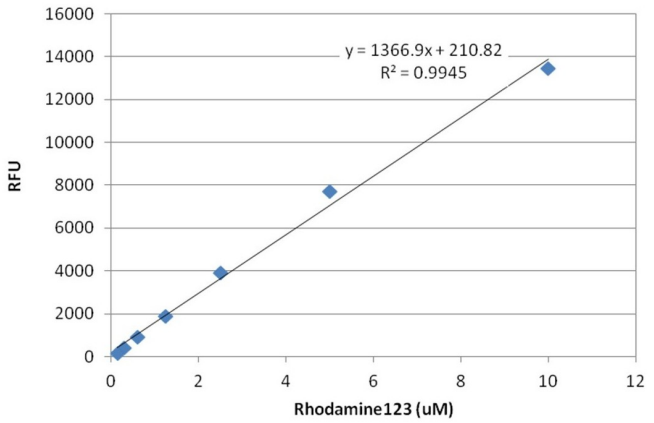

Figure 1. Rhodamine 123 dose response was measured with Amplite™ Fluorimetric LOX Activity Assay Kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em=490 nm/530 nm (cutoff= 515 nm).

Calculations:

1. Subtract the BL (zero Rhodamine 123) RFU reading from all other standard RFU readings.
2. Plot the corrected standard RFU readings against the Rhodamine 123 standards concentration and prepare a standard curve.
3. Subtract the BL control RFU values from LOX sample RFU values.
4. Calculate amount of Rhodamine 123 generated in each LOX reaction using the standard curve

LOX activity in sample (nmol/(min × mL) or mU/mL) = ΔuM/ΔT
ΔuM: Concentration change in uM between time T1 and T2
ΔT: T2 – T1 (minutes)

DISCLAIMER

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