CREATIVE BIOMART®
Assay Kit

Lipoxygenase Activity Assay Kit (Fluorometric)

Product Information

Cat.No. Kit-1061

Product Overview

In Lipoxygenase (LOX) activity assay, lipoxygenase converts the LOX substrate to an intermediate that reacts with the probe generating a fluorescent product. The increase in fluorescent signal can be recorded at Ex/Em 500/536 nm and is directly proportional to LOX activity. The kit includes 5-lipoxygenase enzyme (LOX Enzyme) as a positive control. A lipoxygenase inhibitor that completely inhibits lipoxygenase activity is also included in order to calculate the specific activity of LOX in biological samples. The kit can detect as low as 0.004 mU/mg protein.

Applications

Measurement of Lipoxygenase activity in cell / tissue lysates and serum using a 96-well plate format.

Storage

Upon arrival, store the kit at -20°C. Components are stable for at least three months.
- LOX Buffer and LOX Lysis Buffer: Thaw at RT before use. Keep Lysis Buffer on ice.
- Oxidized Probe Standard: Oxidized probe standard is 100X. Aliquot and store at -20°C in the dark.
- LOX Probe and LOX Inhibitor: Aliquot and store at -20°C in the dark.
- LOX Substrate: Store at -20°C in the dark.
- LOX Enzyme: Aliquot and store at -20°C. Avoid repeated freeze thawing of the aliquots.

Note: Store all kit components on ice while performing the assay.

Size

100 assays

Kit Components

LOX Assay Buffer; 25 ml
Oxidized Probe Standard (100 µM); 200 µl
LOX Probe; 220 µl
LOX Substrate; 6 µl

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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LOX Inhibitor; 100 µl
LOX Enzyme; 22 µl
LOX Lysis Buffer; 2 ml

Materials Required but Not Supplied

Multiwell spectrophotometer capable of reading fluorescence
White 96-well plate
Deionized water
DMSO (anhydrous)

Compatible Sample Types

Cell lysate (i.e., MCF-7 or other cell types expected to have high lipoxygenase activity)
Biological fluids eg. serum
Recombinant enzyme
Purified Protein

Assay Protocol

1. Sample preparation: Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl ice-cold LOX Lysis buffer and keep on ice for 10 minutes followed by centrifugation at 10,000 g for 15 minutes at 4°C. Collect the supernatant and estimate protein concentration using preferred method. We recommend BCA protein assay kit (protein concentration should range between 1 and 10 µg/ µl). Dilute the sample if needed using LOX Assay Buffer. Keep the white 96-well plate on ice while preparing for the assay. Prepare three wells for each sample labelled "Sample Background Control" (BC), "Sample" (S) and "Sample + Inhibiter" (SI). Add 2-10 µl Sample into each of these wells. For SI well add 2 µl LOX Inhibitor in addition to Sample. Adjust the volume in each well to 30 µl with LOX buffer. Several dilutions of the Sample may be tested. For positive control, add 2-4 µl of LOX enzyme into the desired well. Adjust volume to 30 µl with LOX Assay Buffer.
Notes: a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
b. For Samples exhibiting significant background, prepare parallel sample well(s) as Background Controls.
2. Standard Curve generation: Thaw one aliquot of the 100 µM Oxidized Probe Standard at a time

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before performing the assay and prepare working solution by diluting 1:100 with DMSO to obtain 1 µM solution. Add 0, 2, 4, 6, 8, and 10 µl of the 1 µM working solution of the Oxidized Probe Standard in to each well of a white 96-well plate to get 0, 2, 4, 6, 8 and 10 pmol of oxidized probe per well respectively. Make up the volume to 100 µl with LOX buffer. A fresh Standard Curve should be generated each day.

3. LOX Substrate Dilution: Dilute provided LOX substrate immediately before performing the assay by adding 2 µl per 1 ml of LOX Buffer to prepare 1X solution (20 µl/well). Make up enough depending on the number of reactions. Final working solution should be kept on ice and be used up within 2-3 hr. Store the remaining stock solution at -20°C immediately.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. Add BC Mix to "Sample Background Control" wells and Reaction Mix to all other wells. For each well, prepare 70 µl :

BC MIx Reaction Mix

LOX Buffer 68 µl 48 µl

LOX Probe 2 µl 2 µl

LOX Substrate 1X - 20 µl

Add the reaction mix to wells of a 96-well white plate (placed on ice) containing the Samples and Positive Control Note: Have the plate reader ready at Ex/Em 500/536 nm in kinetic mode set to record fluorescence every 30 secs.

5. Measurement: Immediately, start recording fluorescence at 30 second intervals for 30-40 min. Note: Incubation time depends on the LOX activity in Samples. We recommend measuring the OD in kinetic mode and choosing two time points (t1 & t2) in the linear range to calculate the enzymatic activity of the Samples. The oxidized probe Standard Curve can be read in Endpoint mode (i.e. at the end of the incubation time)

6. Calculation: Subtract the Standard Background from Standard Readings and Sample Background Control RFU values from the Sample RFU values respectively. Estimate amount of oxidized probe in the reaction using the Standard Curve. Calculate ΔM, which is the change in amount of oxidized probe between time t1 and t2. LOX Activity may be calculated using the following equations:

Detected activity = ΔM / (Δt x V) x D (nmol/(min x ml) = mUnits / ml)

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Where: ΔM = linear change in oxidized probe concentration during Δt (pmol)
Δt = t2 – t1 (min)
V = Sample protein content added to well (mg)
D = dilution factor
Specific lipoxygenase activity in Sample = detected activity in S – detected activity in SI
Unit Definition: One unit of lipoxygenase is the amount of enzyme that will cause oxidation of 1 µmol of the LOX probe per minute at pH 7.4 at RT.

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