Technical Bulletin

Lipoxygenase Activity Assay Kit

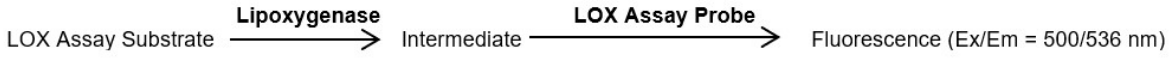
Catalog Number MAK363

Product Description

Lipoxygenases (LOX) are non-heme iron-containing dioxygenases. These enzymes convert unsaturated fatty acids to epoxides; for example, the synthesis of leukotrienes from arachidonic acid is mediated by lipoxygenases. They play an important role in cell proliferation, differentiation, and inflammation, and have been implicated in inflammation and hyperproliferation-mediated diseases like asthma, rheumatoid arthritis, and cancer.

In the Lipoxygenase Activity Assay Kit, lipoxygenase converts the LOX substrate to an intermediate that reacts with the probe, generating a fluorescent product. The increase in fluorescent signal can be measured at λ_{Ex} = 500 nm/λ_{Em} = 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.

The kit is suitable for the measurement of lipoxygenase activity in cell and tissue lysates, biological fluids (for example serum), recombinant enzymes, and purified protein.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- LOX Assay Buffer 25 mL
  Catalog Number MAK363A
- Oxidized Probe Standard (100 µM) 200 µL
  Catalog Number MAK363B
- LOX Probe 200 µL
  Catalog Number MAK363C
- LOX Substrate 6 µL
  Catalog Number MAK363D
- LOX Inhibitor 100 µL
  Catalog Number MAK363E
- LOX Enzyme 40 µL
  Catalog Number MAK363F
- LOX Lysis Buffer 2 mL
  Catalog Number MAK363F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- White flat bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Fluorescence multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1 or equivalent)
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855 or equivalent)
- Reagent Alcohol (200 proof ethanol) (Catalog Number 277649 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Keep components on ice during assay.

LOX Assay Buffer and LOX Lysis Buffer: Warm to room temperature prior to use.

Oxidized Probe Standard: Oxidized probe standard is 100×. Aliquot and store at -20 °C, protected from light.

LOX Probe and LOX Inhibitor: Aliquot and store at -20 °C, protected from light.

LOX Enzyme: Aliquot and store at -20 °C. Avoid repeated freeze/thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize cells (4 × 10^5 cells) or tissue (10 mg) with 100 µL of ice-cold LOX Lysis Buffer and keep on ice for 10 minutes.
2. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
3. Collect the supernatant.
4. Determine protein concentration using preferred method (BCA protein determination is recommended).
5. Protein concentration should range between 1 and 10 µg/µL. Dilute the sample if needed using LOX Assay Buffer.
6. For unknown samples, it is recommended to test several doses to ensure the readings are within the Standard Curve range.
7. Keep the white 96-well plate on ice while preparing for the assay.
8. Prepare three wells for each sample labelled "Sample Background Control" (BC), "Sample" (S) and "Sample plus Inhibitor" (SI).
9. Add 2–10 µL sample into each of the three wells.
10. For SI wells add 2 µL of LOX Inhibitor in addition to sample.
11. Adjust the total volume in each well to 30 µL with LOX Assay Buffer.

Positive Control

For positive control, add 4–8 µL of LOX Enzyme into the desired well. Adjust total volume to 30 µL with LOX Assay Buffer.

Standard Curve Preparation

A new standard curve should be generated each day. Thaw one aliquot of the 100 µM Oxidized Probe Standard at a time before performing the assay.

Prepare a 1 µM Oxidized Probe Premix solution by diluting the thawed 100 µM aliquot 100-fold with DMSO. Prepare Oxidized Probe Standards in desired wells of a white 96-well plate according to Table 1.

Table 1.
Preparation of Oxidized Probe Standards

Well	1 µM Premix	LOX Assay Buffer	Oxidized Probe (pmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	2
3	4 µL	96 µL	4
4	6 µL	94 µL	6
5	8 µL	92 µL	8
6	10 µL	90 µL	10

Reaction Mix

1. Prepare 1× LOX Substrate immediately before performing the assay by adding 2 µL of LOX Substrate to 100 µL of 200 proof ethanol. Prepare enough depending on the number of reactions (2 µL/well). 1× LOX Substrate solution should be kept on ice and can be stored at -20 °C for up to one week. Store the remaining stock solution at -20 ºC immediately.
2. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 2.
   a. For each Sample (S) and Sample plus Inhibitor (SI) well, prepare 70 µL of Reaction Mix. Mix well.
   b. For each Sample Background Control (BC) well, prepare 70 µL of BC Mix. Mix well.

Table 2.
Preparation of Assay Reaction Mixes

Reagent	BC Mix	Reaction Mix
LOX Assay Buffer	68 µL	66 µL
LOX Probe	2 µL	2 µL
1x LOX Substrate	–	2 µL

3. Have the plate reader ready at λ_{Ex} = 500 nm/ λ_{Em} = 536 nm on kinetic mode set to record fluorescence every 30 seconds.
4. Add BC Mix to Sample Background Control (BC) wells and Reaction Mix to Samples (S), Samples plus Inhibitor (SI), and Positive Control wells of a 96-well white plate (pre-chilled on ice) containing the samples and positive control.

Measurement

Immediately start recording fluorescence (RFU) at 30 second intervals for 30-40 minutes at room temperature. The total incubation time depends on the LOX activity in samples. It is recommended to measure in kinetic mode and choosing two time points (T_1 and T_2) 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).

Results

1. Subtract the Standard Background (zero Oxidized Probe) RFU reading from all Standard RFU readings.
2. Plot the corrected Standard RFU readings against the Standard Oxidized Probe concentration and prepare a standard curve.
3. Subtract the Sample Background Control (BC) RFU values from the corresponding Sample (S) RFU values.
4. Estimate amount of oxidized probe in each Sample reaction using the standard curve.

5. Calculate ΔM, which is the change in amount of oxidized probe between time T_1 and T_2.
6. LOX Activity may be calculated using the following equations:

LOX activity in sample (nmol/(min × mL) or mU/mL) =

$$\frac{\Delta M \times DF}{\Delta T \times V}$$

where

ΔM = Change in amount of oxidized probe between time T_1 and T_2

ΔT = $T_2 - T_1$ (minutes)

V = Sample protein content added to well (mg)

DF = Dilution factor of Sample

Specific lipoxygenase activity in sample =

Detected activity in Sample –

Detected activity in Sample plus Inhibitor

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 room temperature.

Figure 1.
Typical Oxidized Probe Standard Curve

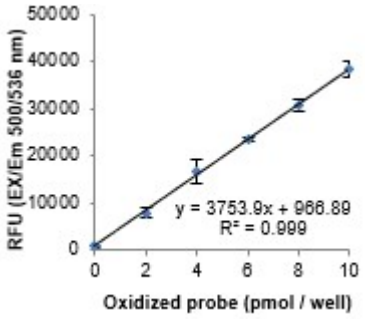


Figure 2.
Lipoxygenase Enzyme Reaction
Lipoxygenase enzyme reaction in positive control and in MCF-7 lysate (10 µg protein).

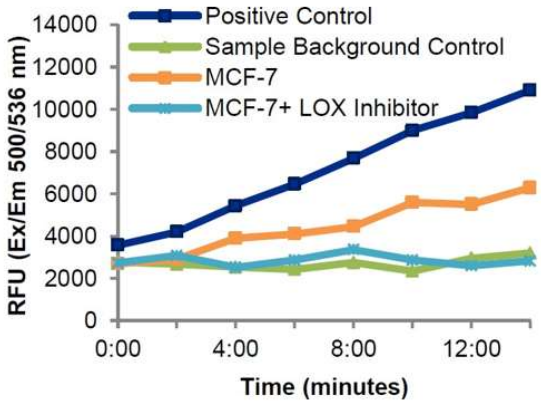
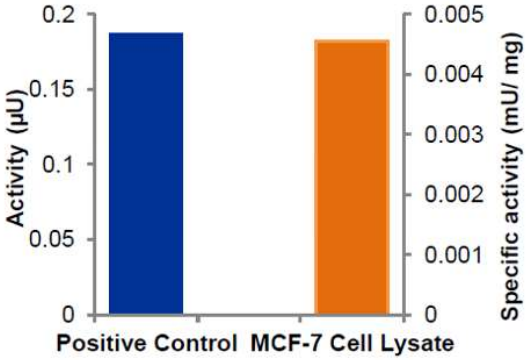


Figure 3.
LOX Enzyme Activity. LOX activity in positive control and MCF-7 lysate. Assays were performed following the kit procedure.



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