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ab241038
Lipoxygenase Assay Kit

For the detection of Lipoxygenase activity in purified protein preparations, recombinant enzymes, tissue lysates, cell lysates, serum and other biological fluids.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

In the Lipoxygenase Assay Kit (ab241038) 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 to calculate the specific activity of LOX in biological samples.

The kit can detect as low as 0.004 mU/mg protein.

2. Protocol Summary

Prepare samples, inhibited samples and positive/background control.

Prepare standard curve.

Prepare reaction mix, then add to standards, positive control and sample wells.

Measure fluorescence (Ex/Em = 500/536 nm) immediately in kinetic mode for 30-40 mins.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
LOX Assay Buffer	25 mL	-20°C
Oxidized Probe Standard (100 µM)	200 µL	-20°C
LOX Probe	200 µL	-20°C
LOX Substrate	6 µL	-20°C
LOX Inhibitor	100 µL	-20°C
LOX Enzyme	40 µL	-20°C
LOX Lysis Buffer	2 ml	-20

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- DMSO

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 LOX Assay Buffer:

Ready to use as supplied. Bring to room temperature before use.

6.2 Oxidized Probe Standard:

Oxidized probe standard is 100X. Aliquot and store at -20°C in the dark. Avoid freeze/thaw.

6.3 LOX Probe:

Ready to use as supplied. Aliquot and store at -20°C in the dark. Avoid freeze/thaw.

6.4 LOX Inhibitor:

Ready to use as supplied. Aliquot and store at -20°C in the dark. Avoid freeze/thaw.

6.5 LOX Substrate:

Ready to use as supplied. Store at -20°C in the dark.

6.6 LOX Enzyme:

Ready to use as supplied. Aliquot and store at -20°C in the dark. Avoid freeze/thaw.

6.7 LOX Lysis Buffer:

Ready to use as supplied. Bring to room temperature before use. Keep on Ice.

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

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 Thaw one aliquot of the 100 µM Oxidized Probe Standard at a time before performing the assay and prepare working solution by diluting 1:100 with DMSO to obtain 1 µM solution.

7.2 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.

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

8. Sample Preparation

8.1 Homogenize cells (4 x 10^5cells) 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 *x g* for 15 minutes at 4°C.

8.2 Collect the supernatant and estimate protein concentration using preferred method; protein concentration should range between 1 and 10 µg/ µL.

8.3 Dilute the sample if needed using LOX Assay Buffer.

Δ Note: Keep the white 96-well plate on ice while preparing for the assay.

8.4 Prepare three wells for each sample labeled 'Sample Background Control' (BC), 'Sample' (S) and 'Sample + Inhibiter' (SI).

8.5 Add 2-10 µL samples into each of these wells.

8.6 For SI well add 2 µL LOX Inhibitor in addition to sample.

8.7 Adjust the volume in each well (BC, S, SI) to 30 µL with LOX buffer.

8.8 For positive control, add 4 - 8 µL of LOX enzyme into the desired well. Adjust volume to 30 µL with LOX Assay Buffer.

Δ Note:

- For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- For samples exhibiting significant background, prepare parallel sample well(s) as background controls.

9. Assay Procedure

LOX Substrate Solution Preparation:

9.1 Dilute the provided LOX substrate immediately before performing the assay by adding 2 µl to 100 µl of 200 proof ethanol (not provided) to generate 1X substrate solution (2 µl/well). Prepare enough depending on the number of reactions. Final working solution should be kept on ice and can be stored at -20 °C up to one week. Store the remaining stock solution at -20 °C immediately.

Reaction Mix Preparation:

9.2 Mix enough reagents for the number of assays to be performed.

9.3 Add BC Mix to 'Sample background control' wells and Reaction Mix to all other wells.

9.4 For each well, prepare 70 µL:

	BC Reaction Mix µL	Reaction Mix µL
LOX Buffer	68	66
LOX Probe	2	2
LOX Substrate 1X	---	2

9.5 Add the reaction mix to wells of a 96-well white plate (placed on ice) containing the samples and positive control.

Δ Note: Have the plate reader ready at Ex/Em 500/536 nm on kinetic mode set to record fluorescence every 30 seconds.

9.6 Immediately start recording fluorescence at 30 second intervals for 30 - 40 minutes at room temperature (RT).

Δ Note: Incubation time depends on the LOX activity in samples. We recommend measuring the OD in kinetic mode and choosing two time points (t1 and 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).

10. Data Analysis

10.1 Subtract the standard background from standard readings and sample background control RFU values from the sample RFU values respectively.

10.2 Estimate amount of oxidized probe in the reaction using the standard curve.

10.3 Calculate ΔM, which is the change in amount of oxidized probe between time t1 and t2.

10.4 LOX Activity may be calculated using the following equations:

Detected activity = **ΔM / (Δt x V) x D** = nmol/(min x mL) = mU/mL

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.

11. Typical Data

Typical data provided **for demonstration purposes only**.

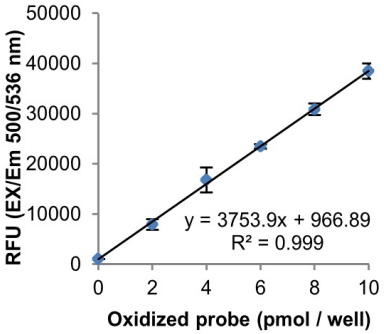

Figure 1. Oxidized probe Standard Curve.

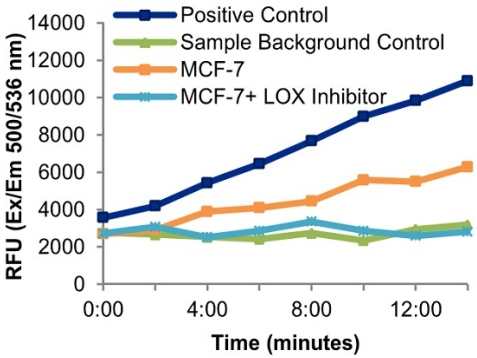

Figure 2. Lipoxygenase enzyme reaction in positive control and in MCF7 lysate (10 µg protein).

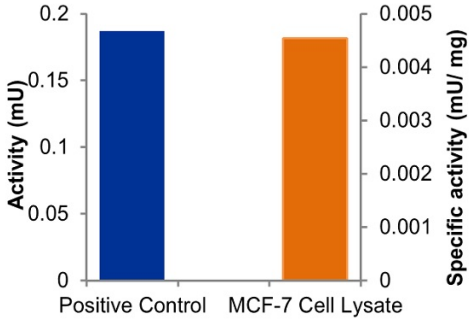

Figure 3. LOX activity in positive control and MCF7 lysate.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)