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ab112139
Lysyl Oxidase (LOX) Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of Lysyl Oxidase (LOX) activity in a variety of samples.

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

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1. Overview

Lysyl Oxidase (LOX) Activity Assay Kit (Fluorometric) (ab112139) provides a simple method to measure lysyl oxidase (LOX) activity in cell and tissue extracts, as well as purified LOX or secreted LOX present in cell culture medium. The assay uses a proprietary LOX substrate that releases hydrogen peroxide upon transformation by the LOX present in the sample. Hydrogen peroxide is in turn detected using our proprietary red fluorescence substrate for HRP-coupled reactions. This leads to increase in fluorescence that can be easily detected at Ex/Em = 540/590 nm in a fluorescence microplate reader.

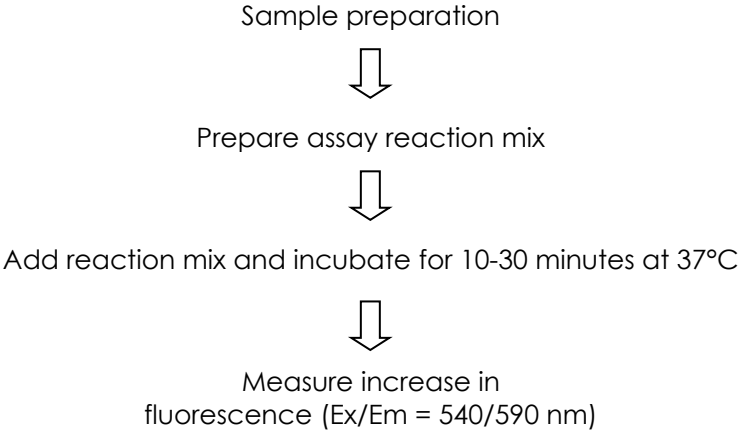
This assay is semi-quantitative as it does not contain a LOX standard for calibration. When a known concentration of LOX is used, the assay can detect activity from as low as 40 ng of lysyl oxidase in solution. The assay is much more sensitive than the other assays currently available in the market and its unique detection method eliminates the interference that occurs in certain biological samples.

Lysyl oxidase (protein-lysine-6-oxidase, LOX, EC 1.4.3.13) is an extracellular copper-dependent enzyme that catalyzes formation of aldehydes from lysine residues in collagen and elastin precursors. These aldehydes are highly reactive and undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues or with unmodified lysine residues. The chemical reactions result in cross-linking collagen and elastin, which is essential for stabilization of collagen fibrils and for the integrity and elasticity of mature elastin.

Lysyl oxidase inhibition can cause osteolathyrism, while its upregulation by tumor cells may promote metastasis of an existing tumor, which makes LOX and important oncological target.

The activity of lysyl oxidase in biological samples is traditionally assessed by tritium release end-point assays using radio isotope labeled collagen or elastin substrates.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer*	50 mL	-20°C	-20°C / 4°C
DMSO	200 µL	-20°C	-20°C
Horseradish peroxidase (lyophilized, 50 U)	1 vial	-20°C	-20°C
HRP Substrate (lyophilized)	1 vial	-20°C	-20°C

*Contains LOX substrate.

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 540/590 nm
- Double distilled water (ddH_2O)
- PBS
- (Optional) BSA – to prepare PBS + 0.1% BSA solution to dilute samples and/or standards.
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96-well plate with clear flat bottom, preferably black
- Active Lysyl Oxidase (LOX) protein (LOX, LOXL1, LOXL2, LOXL3 or LOXL4) to use as standards or control
- BCA Protein Quantification Kit (ab102536) – to determine protein concentration of tissue sample

For cell lysate preparation:

- Cell scraper (for adherent cells)
- RIPA Buffer without DTT or detergents [50 mM Tris pH 8.0, 150 mM NaCl]
- (Optional – if not using PBS) Mammalian Cell Lysis Buffer 5X (ab179835): for lysis of mammalian cells

For tissue sample preparation:

- Extraction Buffer: 6 M urea, 10 mM Tris pH 7.4, protease inhibitors (1 mM PMSF, 1 µM pepstatin A, 6 µM leupeptin)
- Glass beads
- Bead beater

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer (50 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C or 4°C.

9.2 DMSO (200 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Store at -20°C protected from light.

9.3 Horseradish Peroxidase (lyophilized, 50 Units):

Dissolve Horseradish Peroxidase (HRP) in 1 mL of Assay Buffer (see Step 9.1) and mix thoroughly by pipetting up and down. Label this component **50 U/mL HRP Stock Solution**. Keep on ice during the assay. Aliquot Stock HRP solution so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within two months.

9.4 HRP Substrate (lyophilized):

Dissolve HRP substrate in 100 µL DMSO (see Step 9.4) and mix thoroughly by pipetting up and down. Label this component **250X HRP Substrate Stock Solution**. Keep on ice during the assay. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze-thaw cycles. Use within two months.

10. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample.
- Do not use assay buffer to dilute samples as it might cause background. Use PBS + 0.1% BSA to dilute samples.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Δ Note: Presence of detergents (such as Brij-35, Tween-20 and NP-40) or NADH and NADPH will interfere with the assay.

Δ Note: HRP Substrate is unstable in the presence of thiols such as DTT, reduced glutathione (GSH) and β-mercaptoethanol. Presence of thiols at a concentration > 10 µM will significantly decrease the assay dynamic range.

10.1 Cell lysates:

Δ Note: For ease of use, mammalian adherent or suspension cells lysates can be easily prepared using Mammalian Cell Lysis Buffer 5X (ab179835). Follow product protocol and proceed to Assay Procedure Section.

10.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 2-5 x 10^5 cells).
10.1.2 Wash cells with cold PBS.
10.1.3 Resuspend or scrape cells in 100 µL of cold PBS (or RIPA buffer without DTT or detergents).
10.1.4 Homogenize cells quickly by pipetting up and down a few times.
10.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.
10.1.6 Collect supernatant and transfer to a new tube.
10.1.7 Keep on ice.
10.1.8 Determine protein concentration of your sample.
10.1.9 Prepare multiple dilutions of sample in PBS + 0.1% BSA.

10.2 Tissue lysates:

10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 1-2 mg).
10.2.2 Wash tissue in cold PBS.
10.2.3 Place tissue into a 1.5 mL homogenizer tube (eg BeadBeater tube) pre-loaded with glass beads, sitting on ice.
10.2.4 Add 0.5-1 mL Extraction Buffer (6 M urea, 10 mM Tris pH 7.4 and protease inhibitors) to the homogenizer tube.
10.2.5 Homogenize the sample in the homogenizer tube for 90 seconds, then place on ice again.
10.2.6 If sample is not completely homogenized, repeat previous step.
10.2.7 Centrifuge sample for 5-10 minutes at 4°C at 13,000 x*g* using a cold microcentrifuge to remove any insoluble material.
10.2.8 Collect supernatant and transfer to a new tube.
10.2.9 Keep on ice.
10.2.10 Determine protein concentration of your sample.
10.2.11 Prepare multiple dilutions of sample in PBS + 0.1% BSA.

10.3 Cell or tissue culture supernatant:

Δ Note: The presence of serum in the culture medium does not interfere with the assay.

10.3.1 Collect culture supernatant.
10.3.2 Centrifuge samples at 13,000 x*g* for 2-5 minutes at 4°C in a cold centrifuge.
10.3.3 Transfer supernatant to a new tube.
10.3.4 Keep on ice.
10.3.5 Prepare multiple dilutions of sample in PBS + 0.1% BSA.

10.4 Purified protein:

Use directly or dilute in PBS + 0.1% BSA.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- This kit does not contain active LOX standard. This component needs to be provided by the user.
- The protocol describe in this section is for 1 x 96-well plate. To perform the assay in a 384-wp, scale down volumes by 50%.

Δ Note: HRP Substrate is unstable in the presence of thiols such as DTT, reduced glutathione (GSH) and β-mercaptoethanol. Presence of thiols at a concentration > 10 µM will significantly decrease the assay dynamic range.

Δ Note: High concentration of LOX may cause reduced fluorescence signal due to the overoxidation of the HRP substrate to a non-fluorescent product.

11.1 (Optional) LOX standard control preparation:

Δ Note: LOX standard are used for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

11.1.1 Dilute LOX protein stock solution in PBS + 0.1% BSA to prepare a 10 µg/mL stock solution.

11.1.2 Using the 10 µg/mL stock solution, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to dilute	Volume standard in well (µL)	PBS + 0.1% BSA (µL)	End conc LOX in well
1	10 µg/mL	150	225	4 µg/mL
2	Std #1	150	225	1.6 µg/mL
3	Std #2	150	225	0.64 µg/mL
4	Std #3	150	225	0.256 µg/mL
5	Std #4	150	225	0.102 µg/mL
6	Std #5	150	225	0.04 µg/mL
7 (Blank)	0	0	200	0 µg/mL

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11.2 Set up reaction wells:

- Blank control = 50 µL Assay Buffer.
- Standard wells = 50 µL LOX standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with sample preparation buffer).

Δ Note: Include non-treated samples in your assay as control

See example plate layout for assay in the table below:
BL: Blank Control
TS: test sample wells (including non-treated and treated samples)
LS: Lysyl oxidase standards

BL	BL	TS	TS	…	…		
LS1	LS1						
LS2	LS2						
LS3	LS3						
LS4	LS4						
LS5	LS5						
LS6	LS6						
LS7	LS7						

11.3 LOX reaction mix:

11.3.1 Prepare 2x LOX Reaction Mix as described in the table below. The volume given in the table is enough for 1 x 96-well plate.

Component	Reaction Mix
250X HRP Substrate Stock Solution	20 µL
50 U/mL HRP Stock Solution	20 µL
Assay Buffer	5 mL
TOTAL VOLUME	5.040 mL

11.4 LOX assay procedure:

11.4.1 Add 50 µL of LOX Reaction Mix into each well to make the total assay volume of 100 µL.

11.4.2 Mix and incubate at 37°C for 10-40 min protected from light.

11.4.3 Monitor fluorescence increase on a microplate reader at Ex/Em = 530-570/590-600 nm (maximum Ex/Em = 540/590 nm, cut off as 570 nm).

Δ Note: this assay is run on a 96-well black wall/clear bottom plate. The contents of the plate can also be transferred to a white clear bottom plate and read by a colorimetric microplate reader at OD = 576 ± 5 nm. The colorimetric detection has lower sensitivity compared to fluorescence reading.

12. Data Analysis

– Subtract the blank control (assay buffer only) from all wells containing LOX (samples and standard, if using).

Δ Note: fluorescence background increases with time; therefore, it is important to subtract the fluorescence intensity value of the blank wells for each data point.

– Calculate the mean value of the duplicate readings for each standard and sample.
– Using fluorescent intensity, determine fold change between un-treated (control) and treated sample.
– If you have quantified the protein in the sample, LOX activity levels can be shown relatively to the amount of protein present in the sample and incubation time (µg/mL/min).

Δ Note: LOX standards in this assay are used for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

13. Typical Data

Data provided **for demonstration purposes only.**

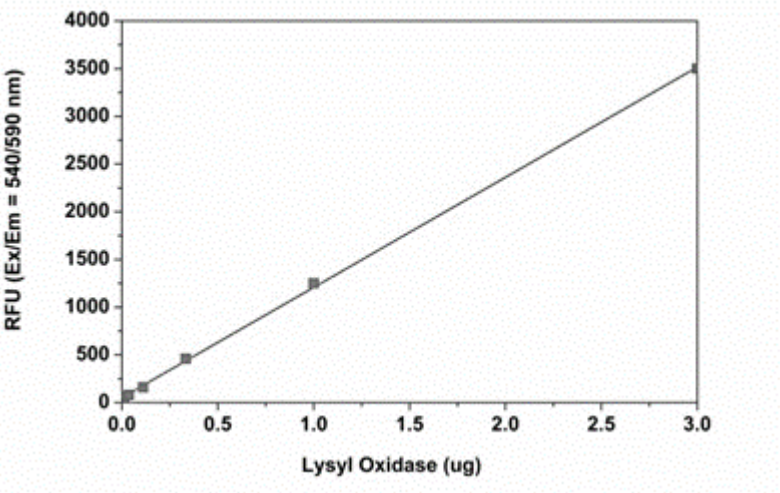


Figure 1. Typical lysyl oxidase (LOX) dose response curve. Known amounts of LOX were added to wells and reaction was run following assay protocol. Fluorescence was measured on a solid black 96-well plate using a Gemini fluiorescence microplate reader (Molecular Devices). Activity from as low as 40 ng/mL of LOX can be detected after 30 minutes incubation.

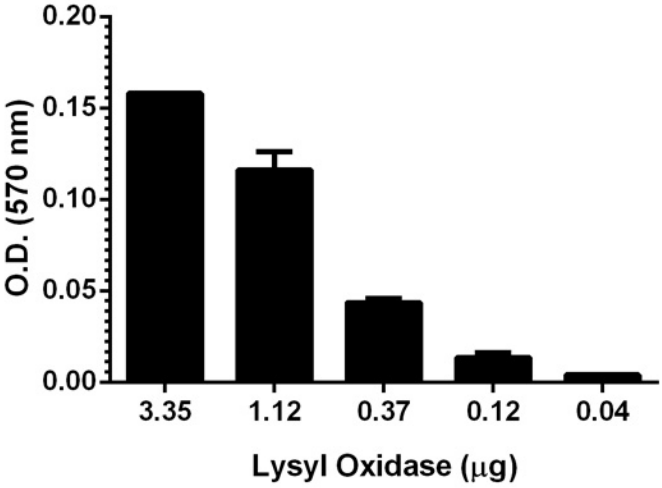


Figure 2. LOX activity absorbance (OD) values vs quantity (µg). Recombinant human LOX2 was serially diluted 3.35-0.04 (1/3) and activity was measured following assay procedure. Colorimetric detection is approximately 10-times lower than fluorometric.

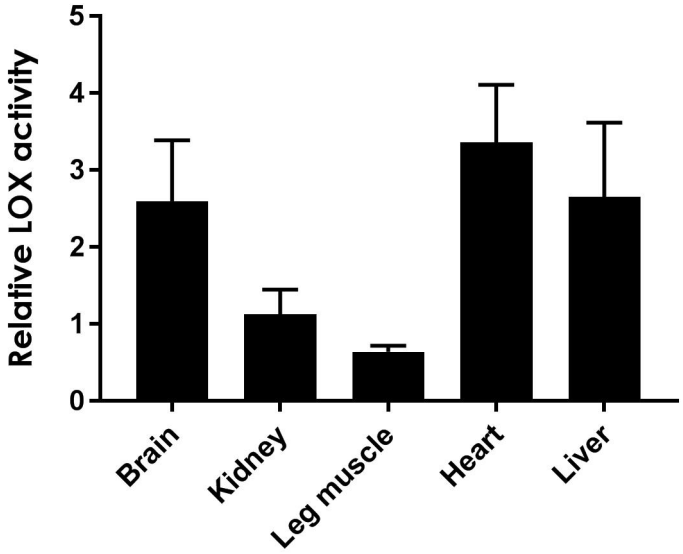


Figure 3. Relative LOX activity levels in mouse tissue. Tissue samples were prepared following assay protocol. Protein concentration was determined and samples were diluted 2-30 fold. LOX activity levels were measured after 15 minutes incubation in a fluorometric plate reader at Ex/Em = 535/587 nm. LOX activity levels are relative to background noise (blank control).

14. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

15. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Detergents Brij-35, Tween-20, NP-40.
- NADH and NADPH
- Thiols (DTT, GSH, β-mercaptoethanol) > [10 µM]

16. FAQs

Q. How can I use BAPN to study LOX activity?

A. Beta-aminopropionitrile (BAPN) is a LOX inhibitor that can be used to perform inhibitory studies.

An inhibitory assay can be performed following the Assay Procedure but we recommend setting up the following controls:

- Blank Control: sample diluent + 1 µL BAPN solvent (ddH_2O)
- Negative control: sample diluent + 1 µL 10 mM BAPN
- Positive control: sample + 1 µL ddH_2O

For inhibitory studies, we recommend reading the fluorescence increase in kinetic mode.

17. 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)