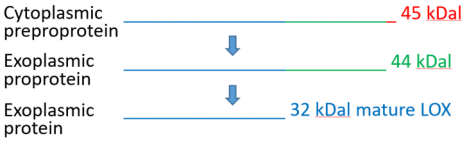
V. 1.0

Lysyl Oxidase (32 kDal form)
(Bovine Leg Tendon)
Cat. # CS-LXE01, 1 x 5.0 µg
Lot #
Upon arrival store at 4°C (desiccated)

Material

Lysyl oxidase (LOX, Acc.# NM_173932.4, similar to Human Acc.# NM_001178102, EC 1.4.3.13) is a copper containing 32 kDal protein found in structural tissues where it functions to promote crosslinking of structural proteins resulting in higher tensile strength of the biopolymer. Although concentrations are up to 500 µg/ml in some tissues (e.g. tendon), the yields are very low in the purified form, usually 1-2 mg per Kg of raw material (Kagan and cai, 1995). LOX is supplied as a white lyophilized powder.

Figure 1. Mature LOX 32 kDal is a product of a 45 kDal pre-proprotein of LOX.



Legend: LOX preproprotein is cleaved on its exit from the cell, which makes the proprotein which is subsequently proteolytically cleaved to form the mature active LOX enzyme.

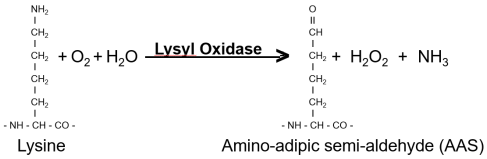


Figure 2. Reaction of LOX with oxygen and lysine residues.

Legend: LOX will exchange the NH2 group with O, which creates a reactive entity for crosslinking to other tertiary amines. In the assays of LOX, H2O2 is used to oxidize reporter molecules with horse radish peroxidase as a catalyst.

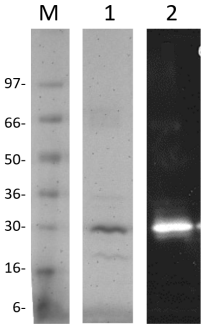
Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a 5 µg tube of CS-LXE01 with 100 µl of nanopure will generate a 50 µg/ml stock of LOX in the following buffer: 4M urea in PBS pH 7.4. The protein can be frozen in liquid nitrogen and stored at –70°C. It is not recommended to store at 4°C in liquid form. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 6 months.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% gradient polyacrylamide gel. LOX was determined to be >80% pure (see Figure 3).

Figure 3. LOX analysis by SDS-PAGE and antibody identification.



Legend: A 2 µg sample of LOX (lane 1) separated by electrophoresis using a 4-20% SDS-PAGE gel and stained with Coomassie Blue. In Lane 2, the immunoblotted protein was probed with anti -LOX (NBP100-2527) Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). SeeBlue molecular weight markers are from Life Technologies Inc.

Biological Activity Assay

LOX is assayed using a physiological lysine containing substrate, lysine or poly-lysine are not good substrates. Collagen, elastin and putrescine or cadaverine are good substrates. Urea is a necessary solubilizing agent for LOX, but above 1M it also inhibits its activity. Lox readily precipitates and cross links with other proteins and itself, so it is a good strategy to store in 4M urea, which effectively stalls its activity, and dilute into the reaction when other components are ready. Beta-aminopropionitrile (BAPN) is a good control inhibitor (Rodriguez, et al. 2008b).

Reagents

1. LOX protein (1 x 5 µg; Cat. # LXE01)
2. Elastin or Collagen (1 mg/ml in PBS), or 2M putrescine or cadaverine in dry (fresh vial) DMSO. (OPD format only)
3. 4M urea in PBS pH 7.4 (fluorescence), or nanopure water (OPD).
4. PBS pH 7.4 plus 0.1% (w/v) bovine serum albumin (BSA).
5. 100 mM Borate pH8.0, 100 mm NaCl.
6. EITHER OPD tablets (Sigma Cat.# P9187-5SET) and HRP enzyme (P8375).
7. OR Fluorescence assay kit (Abcam Cat.# ab112139)
8. 10x concentrated compounds in PBS.

Equipment

1. Spectrophotometer capable of measuring absorbance at 650 nm (+/- 20 nm bandwidth) for OPD method, or 540nm/590nm Ex/Em fluorescence for fluorescence method.
2. Half area 96 well microtiter plate, clear (Corning Cat.# 3696 or 3697) for OPD method, or half area black (Corning cat.# 3915) for fluorescence method.
3. Multi-channel pipette.

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Method

The following major steps are recognized:

Step 1. Assemble required reagents and compounds, and set up plate reader (30 min).
Step 2. Solubilize LOX on ice (5 min).
Step 3. Prepare LOX reaction mix (5 min).
Step 4. Pipette compound into well.
Step 5. Pipette LOX into well.
Step 6. Pipette reaction mix into wells and start incubation (up to 6 hours).
Step 7. Read results in plate reader (10-30 min).

Fluorescence method

Note: This method is sensitive to approx. 5 ng/ml of LOX enzyme.

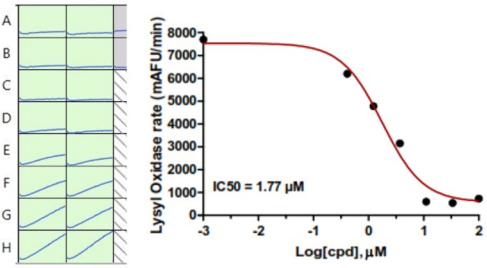
1. Place LOX vial on ice.
2. Resuspend LOX with 500 µl of 4M urea in PBS buffer.
3. Make LOX reaction mixture:
   625 µl Reaction Buffer from Abcam kit.
   2.5 µl HRP substrate from kit.
   2.5 µl HRP enzyme from kit.
5. Using the clear half area 96-well plate, pipette the following in duplicate per sample:
   - 10 µl of compound (if required).
   - 10 µl of LOX.
   - 50 µl of PBS pH 7.4 plus 0.1% BSA.
   - 50 µl of LOX reaction mix.
6. Place in plate reader at 37°C and read 61 times with 30s intervals.
7. Subtract buffer only control.

OPD method

Note: This method is approx. 100x less sensitive than the fluorescence method, it can only detect the highest levels of LOX protein e.g. >=5 µg/ml levels.

1. Prepare 2x strength OPD "silver pouch" tablet.
2. Place LOX vial on ice.
3. Resuspend LOX with 100 µl of nanopure water.
4. Make LOX reaction mixture:
   666 µl of 100 mM Borate pH8.0, 100 mm NaCl.
   333 µl 2x strength Sigma "silver pouch" tablet.
   1 µl 2M Putrescine stock in dry DMSO.
   1 µl HRP (1 u/ml)
5. Using the clear half area 96-well plate, pipette the following in duplicate per sample:
   - 10 µl of compound (if required).
   - 10 µl of LOX.
   - 50 µl of PBS pH 7.4 plus 0.1% BSA.
   - 50 µl of LOX reaction mix.
6. Incubate at 37°C for 4 h.
7. Read at 650nm, and subtract buffer only control.

Figure 4: Assay of LOX with dose response curve due to BAPN inhibition



Legend: The LOX assay was set up as described in the fluorescence protocol above with BAPN titrated between 0 and 100 µM . Mini-kinetic graphs are presented on the left, and the results plotted on a dose response graph on the right. IC50 = 1.77 µM as calculated with a four parameter Log equation from Prizm software.

Product Uses

- Measurement of LOX inhibition by compounds.
- Positive control in immunodiagnostics.
- Positive control as LOX enzyme in comparisons to LOXL enzymes.
- Identification/characterization of substrates of LOX

References

1. Kagan and Cai, 1995. Isolation of Active Site Peptides of Lysyl Oxidase. Metods in Enzymology, 258, p122-132.
2. Rodríguez, C. et al. 2008b. Lysyl oxidase as a potential therapeutic target. Drug News Perspect. 21, 218-224. doi:10.1358/dnp.2008.21.4.1213351.

Product Citations/Related Products

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