

Product Datasheet

LOX Antibody NB100-2530SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Publications: 6

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NB100-2530SS

LOX Antibody

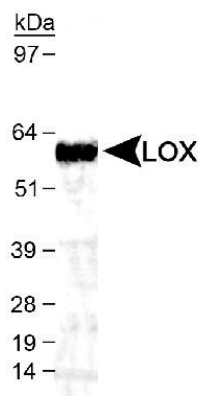
Product Information	
Unit Size	0.025 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Ammonium sulfate precipitation
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	4015
Gene Symbol	LOX
Species	Human, Mouse, Rat, Bovine, Porcine
Species Reactivity	Human, mouse, rat, cow and pig. 92% sequence identity with chicken, Zebrafish, and Xenopus proteins.
Immunogen	A cocktail of two synthetic peptides; one made to a region of the human LOX protein within residues 300-350 (NB100-2528) and one within residues 200-300 (NB100-2527). [Swiss-Prot P28300]

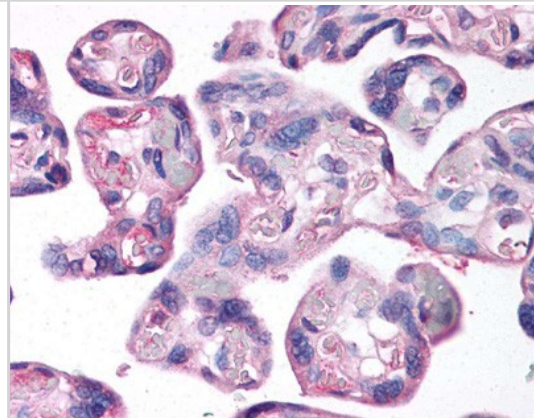
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200, Western Blot 1:1000-1:5000, Simple Western 1:100
Application Notes	This LOX antibody is useful for Western blot and Immunohistochemistry paraffin embedded sections. In Western blot a band is seen at ~58 kDa. This is a highly glycosylated protein; therefore one may see bands at ~32 kDa, ~50 kDa and ~58 kDa representing the mature (secreted) form, the pro form and the glycosylated forms respectively. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

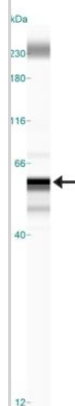
Western Blot: LOX Antibody [NB100-2530] - Detection of LOX in mouse kidney lysate using NB 100-2530 (1:1,000). ECL detection was 15 sec.



Immunohistochemistry: LOX Antibody [NB100-2530] - Human placental villi, 40X. Staining of placental trophoblasts using NB100-2530 at 10 ug/ml.



Simple Western: LOX Antibody [NB100-2530] - Simple Western lane view shows a specific band for Lox in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Sethi A, Wordinger RJ, Clark AF. Gremlin utilizes canonical and non-canonical TGFbeta signaling to induce lysyl oxidase (LOX) genes in human trabecular meshwork cells. *Exp Eye Res* 2013 Jun 5 [PMID: 23748100] (WB, Human)

Isabelle Desguerre MD, PhD, Ludovic Arnold PhD, Alban Vignaud PhD, Sylvain Cuvelier MSc, Houda Yacoub-Youssef PhD, Romain K Gherardi MD, PhD, Jamel Chelly MD, PhD, Fabrice Chretien MD, Remi Mounier PhD, Arnaud Ferry PhD, Benedicte Chazaud PhD. A new model of experimental fibrosis in hindlimb skeletal muscle of adult mdx mouse mimicking muscular dystrophy. *Muscle & Nerve*: 1097-4598.

Mizuno S, Yasuo M, Bogaard HJ et al. Copper deficiency induced emphysema is associated with focal adhesion kinase inactivation. *PLoS ONE* 20127(1):e30678. [PMID: 22276220]

Desguerre I, Arnold L, Vignaud A, Cuvelier S, Yacoub-Youssef H, Gherardi RK, Chelly J, Chretien F, Mounier R, Ferry A, Chazaud B. A new model of experimental fibrosis in hindlimb skeletal muscle of adult mdx mouse mimicking muscular dystrophy. *Muscle Nerve*;45(6):803-14. 2012 Jun. [PMID: 22581532] (IHC, Mouse)

Sethi A, Mao W, Wordinger RJ, Clark AF. Transforming Growth Factor Beta Induces Extracellular Matrix Protein Crosslinking Lysyl Oxidase (LOX) Genes in Human Trabecular Meshwork Cells. *Invest Ophthalmol Vis Sci*. 2011 May 5. [PMID: 21546528]

Horiguchi M, Inoue T, Ohbayashi T et al. Fibulin-4 conducts proper elastogenesis via interaction with cross-linking enzyme lysyl oxidase. *Proc Natl Acad Sci U S A*;106(45):19029-34. 2009 Nov 10. [PMID: 19855011]



Procedures

Western Blot Protocol for LOX Antibody (NB100-2530)

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH₂O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 2 hours at room temperature.
6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-LOX primary antibody (NB 100-5230) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Pierce's ECL). Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

IHC-FFPE sections

I. Deparaffinization:

- A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

- A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
- B. Place slides in distilled water: 2 changes for 2 minutes each.



III. Retrieve Epitopes:

- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celsius.
- B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
- C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
- D. Slowly add distilled water to further cool for 5 minutes.
- E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

- A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap-Pen).
- B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
- C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
- D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
- E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
- G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
- I. Wash slides with Wash Solution: 3 changes for 5 minutes each. Wash slides with Wash Solution: 3 changes for 5 minutes each
- J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
- K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
- L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
- M. Rinse slides in distilled water.
- N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
- O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
- P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.



Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.

-Prior to deparaffinization, heat slides overnight in a 60 degrees Celsius oven.

-All steps in which Xylene is used should be performed in a fume hood.

-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1





Novus Biologicals USA

8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada

461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.

