

Product Datasheet

LOX Antibody - BSA Free NB100-2530

Unit Size: 0.05 ml

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

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NB100-2530

LOX Antibody - BSA Free

Product Information	
Unit Size	0.05 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

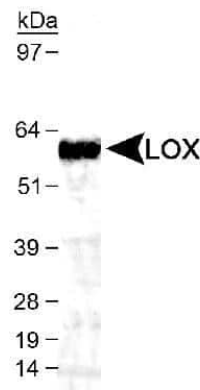
Product Description	
Description	Novus Biologicals Rabbit LOX Antibody - BSA Free (NB100-2530) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and Simple Western. Anti-LOX Antibody: Cited in 17 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	4015
Gene Symbol	LOX
Species	Human, Mouse, Rat, Porcine, Bovine
Reactivity Notes	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-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:500-1:1000, Simple Western 1:100, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:200
Application Notes	<p>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.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 57 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

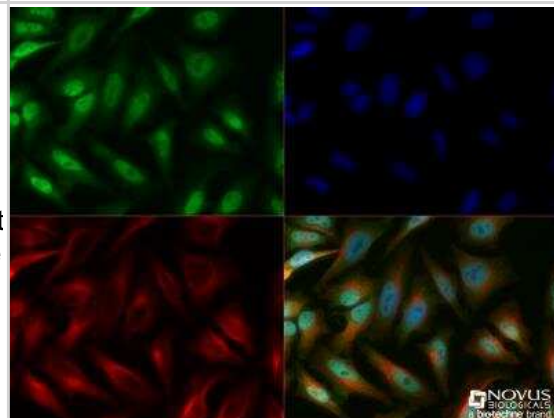


Images

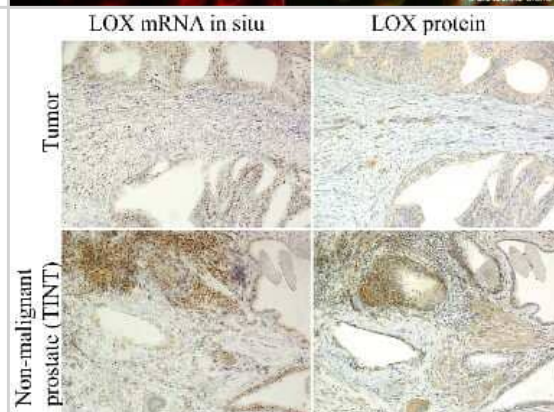
Western Blot: LOX Antibody [NB100-2530] - Detection of LOX in mouse kidney lysate using NB 100-2530 (1:1,000). ECL detection was 15 sec.



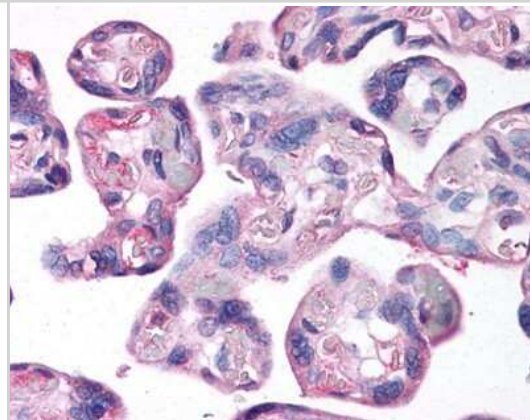
Immunocytochemistry/Immunofluorescence: LOX Antibody [NB100-2530] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-LOX [NB100-2530] at a 1:100 dilution overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



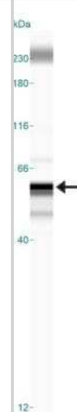
Immunohistochemistry-Paraffin: LOX Antibody [NB100-2530] - Lysyl Oxidase (LOX) expression in malignant and non-malignant human prostate tissue. Consecutive sections from non-malignant prostate tissue stained with in situ hybridization or immunohistochemistry for LOX. Note that mRNA (brown dots) and protein (brown) expression in individual glands were related, in the stroma in contrast mRNA was generally low but protein often detected (original magnifications x200). Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0140985>), licensed under a CC-BY license.



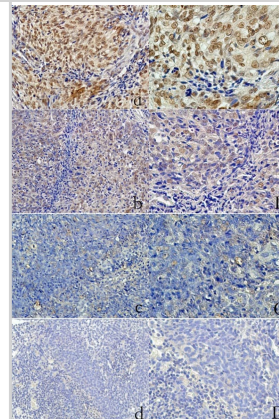
Immunohistochemistry: LOX Antibody [NB100-2530] - Human placental villi, 40X. Staining of placental trophoblasts using NB100-2530 at 10 ug/ml.



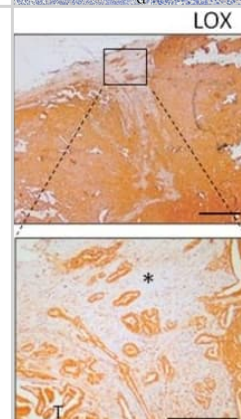
Simple Western: LOX Antibody [NB100-2530] - Simple Western lane view shows a specific band for LOX in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



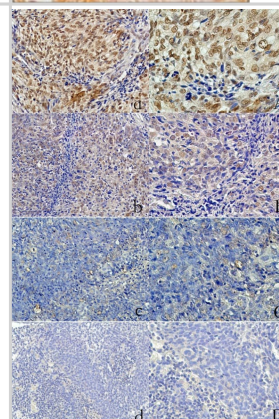
Expression of LOX in primary nasopharyngeal carcinoma (IHC staining)
 Strong expression(a) magnification, $\times 200$; (A) magnification, $\times 400$;
 Moderate expression: (b) magnification, $\times 200$; (B) magnification, $\times 400$;
 Weak expression: (c) magnification, $\times 200$; (C) magnification, $\times 400$;
 Negative: (d) magnification, $\times 200$; (D) magnification, $\times 400$.



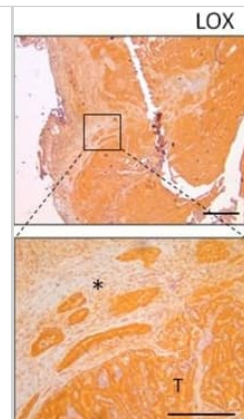
Immunohistochemistry: LOX Antibody - BSA Free [NB100-2530] - α -SMA, LOX, & COL1A1 immunostaining in human thyroid cancers. Representative thyroid tumors serial sections from two different patients stained by IHC for α -SMA, COL1A1, & LOX protein expression & localization. In the top panel, the tumor edge/invasive front is specifically shown (scale bar 500 μ m), while lower panel has higher magnification (scale bar 200 μ m). * clusters of tumor invading cells detaching from the principal tumor mass (T). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31906302>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



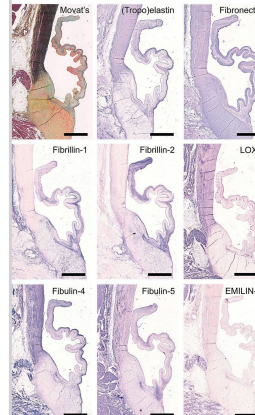
Immunohistochemistry: LOX Antibody - BSA Free [NB100-2530] - Expression of LOX in primary nasopharyngeal carcinoma (IHC staining)
 Strong expression(a) magnification, $\times 200$; (A) magnification, $\times 400$;
 Moderate expression: (b) magnification, $\times 200$; (B) magnification, $\times 400$;
 Weak expression: (c) magnification, $\times 200$; (C) magnification, $\times 400$;
 Negative: (d) magnification, $\times 200$; (D) magnification, $\times 400$. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.6996>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



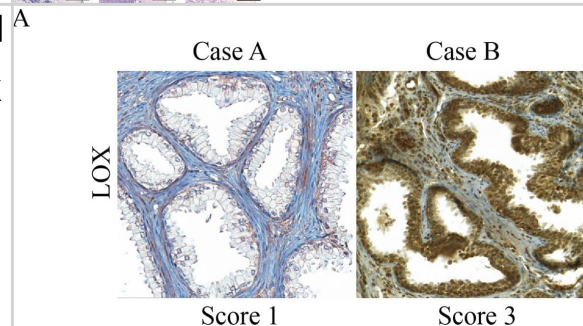
Immunohistochemistry: LOX Antibody - BSA Free [NB100-2530] - α -SMA, LOX, & COL1A1 immunostaining in human thyroid cancers. Representative thyroid tumors serial sections from two different patients stained by IHC for α -SMA, COL1A1, & LOX protein expression & localization. In the top panel, the tumor edge/invasive front is specifically shown (scale bar 500 μ m), while lower panel has higher magnification (scale bar 200 μ m). * clusters of tumor invading cells detaching from the principal tumor mass (T). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31906302>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



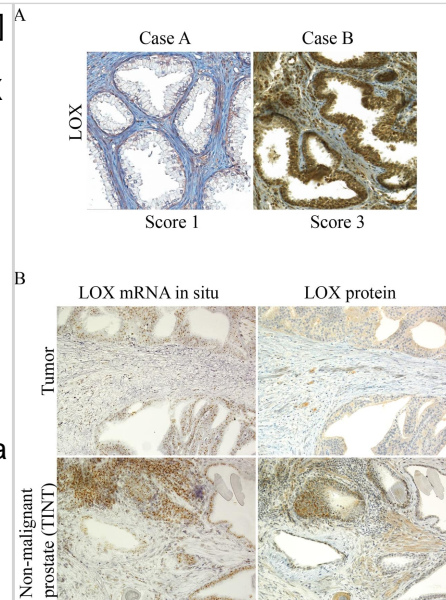
Immunohistochemistry-Paraffin: LOX Antibody - BSA Free [NB100-2530] - Elastin & elastin-related proteins. Tile scans of sheep aortic valve sections stained with Russell Movat's pentachrome (mature elastic fibers in black) & antibodies against proteins involved in elastic fiber formation, including the core protein (tropo)elastin, fibronectin, the microfibrillar proteins fibrillin-1 & fibrillin-2, & cross-linking proteins fibulin-4, fibulin-5, lysyl oxidase (LOX), & Elastin microfibril interfacier 1 (EMILIN-1). Scale bars, 1 mm. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30159315>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



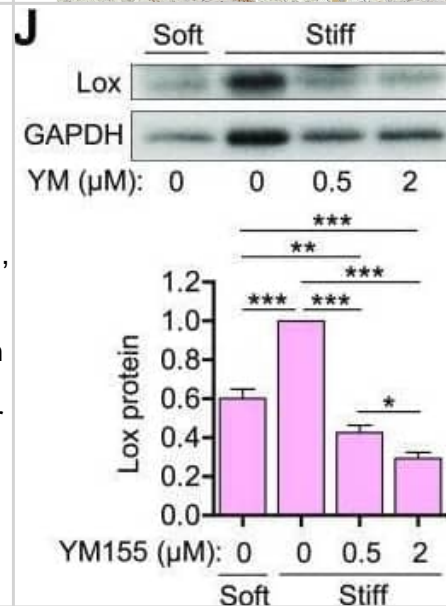
Immunohistochemistry-Paraffin: LOX Antibody - BSA Free [NB100-2530] - Lysyl Oxidase (LOX) expression in malignant & non-malignant human prostate tissue. (A) Representative immunohistochemical staining of LOX (brown) in sections of non-malignant prostate tissue specimens (TINT epithelium & TINT stroma) from two prostate cancer patients (original magnifications x 200). Case A show weak epithelial staining (score 1) & Case B strong epithelial staining (score 3). (B) Consecutive sections from non-malignant prostate tissue stained with in situ hybridization or immunohistochemistry for LOX. Note that mRNA (brown dots) & protein (brown) expression in individual glands were related, in the stroma in contrast mRNA was generally low but protein often detected (original magnifications x200). (C) LOX mRNA expression in non-malignant prostate tissue (TINT) (n = 12), in primary prostate tumor tissue (n = 12), & in castration-resistant bone metastases (CRPC) (n = 30) using Illumina gene expression array. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/26501565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry-Paraffin: LOX Antibody - BSA Free [NB100-2530]
 - Lysyl Oxidase (LOX) expression in malignant & non-malignant human prostate tissue. (A) Representative immunohistochemical staining of LOX (brown) in sections of non-malignant prostate tissue specimens (TINT epithelium & TINT stroma) from two prostate cancer patients (original magnifications x 200). Case A show weak epithelial staining (score 1) & Case B strong epithelial staining (score 3). (B) Consecutive sections from non-malignant prostate tissue stained with in situ hybridization or immunohistochemistry for LOX. Note that mRNA (brown dots) & protein (brown) expression in individual glands were related, in the stroma in contrast mRNA was generally low but protein often detected (original magnifications x200). (C) LOX mRNA expression in non-malignant prostate tissue (TINT) (n = 12), in primary prostate tumor tissue (n = 12), & in castration-resistant bone metastases (CRPC) (n = 30) using Illumina gene expression array. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/26501565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



ECM synthesis in hVSMCs is reduced when survivin expression is suppressed. (a) and (f) hVSMCs were synchronized to G0 by serum starvation and plated on fibronectin-coated soft or stiff hydrogels with 10% FBS for 24 h. (b)–(e) and (g)–(j) Serum-starved hVSMCs were plated on soft or stiff hydrogels with 10% FBS with DMSO or YM155 at the indicated concentrations for 24 h. Levels of mRNA (a)–(e) and protein (f)–(j) were analyzed by RT-qPCR and immunoblotting assays, respectively. The graphs show the expression of survivin (a), (b), and (e), collagen-I (c) and (h), fibronectin (d) and (i), and Lox (e) and (j). Expression was normalized to that in hVSMCs treated with DMSO (vehicle control) on stiff hydrogels. n = 7 (a), n = 3 – 8 (b)–(e), n = 6 (f), n = 4 (g) and (j), n = 3 (h) and (i). Error bars show SEMs. *p < 0.05; **p < 0.01; ***p < 0.001; and ns, not significant by Student's t test (a) and (f) or ANOVA followed by Newman–Keuls post hoc test for multiple comparisons (b)–(e) and (g)–(j). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37868708>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Krajnik A, Nimmer E, Brazzo JA et al. Survivin regulates intracellular stiffness and extracellular matrix production in vascular smooth muscle cells APL bioengineering 2023-12-01 [PMID: 37868708] (WB, Human)

Ballester-Servera C, Alonso J, Cañes L et al. Lysyl oxidase-dependent extracellular matrix crosslinking modulates calcification in atherosclerosis and aortic valve disease Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2023-09-18 [PMID: 37729730] (WB, Human)

Shah KM, Tattersall L, Hussain A, Macfarlane SC P2RX7 inhibition reduces breast cancer induced osteolytic lesions-implications for bone metastasis bioRxiv 2022-01-01 (WB, Mouse)

Seborova K, Hlavac V, Rob L Et al. 707 Non-coding RNA and mRNA transcriptome differences in ovarian carcinoma patients associated with resistance to adjuvant chemotherapy Ovarian cancer 2021-10-01 (WB, IF/IHC)

Yehezkely R, Zaffryar-Eilot S, Kaganovsky A et al. Intracellular Role for the Matrix-Modifying Enzyme Lox in Regulating Transcription Factor Subcellular Localization and Activity in Muscle Regeneration Developmental Cell 2020-05-18 [PMID: 32359406]

Treissman J, Yuan V, Baltayeva J et al. Low oxygen enhances trophoblast column growth by potentiating differentiation of the extravillous lineage and promoting LOX activity Development 2020-01-23 [PMID: 31871275] (WB)

Minna E, Brich S, Todoerti K et al. Cancer Associated Fibroblasts and Senescent Thyroid Cells in the Invasive Front of Thyroid Carcinoma Cancers (Basel) 2020-01-01 [PMID: 31906302] (IF/IHC, IHC-P, Human)

Kim JH, Lee G, Won Y et al. Matrix cross-linking-mediated mechanotransduction promotes posttraumatic osteoarthritis Proc Natl Acad Sci U S A. 2015-07-27 [PMID: 26170306] (WB, ICC/IF, IF/IHC, Mouse)

Dekker S, van Geemen D, van den Bogaerdt AJ et al. Sheep-Specific Immunohistochemical Panel for the Evaluation of Regenerative and Inflammatory Processes in Tissue-Engineered Heart Valves Front Cardiovasc Med 2018-08-15 [PMID: 30159315] (IHC-P, Human)

Yamaguchi Yukie, Takihara Takahisa, Chambers Roger A et al. A peptide derived from endostatin ameliorates organ fibrosis. Science Translational Medicine 2012-01-01 [PMID: 22649092] (IF/IHC, Human)

Hua YJ, Wang HY, Tang LQ et al. LOX expression in primary nasopharyngeal carcinoma: correlation with prognostic parameters and outcome. Oncotarget 2016-02-16 [PMID: 26882568] (IF/IHC)

Nilsson M, Hagglof C, Hammarsten P et al. High Lysyl Oxidase (LOX) in the Non-Malignant Prostate Epithelium Predicts a Poor Outcome in Prostate Cancer Patient Managed by Watchful Waiting. PLoS ONE. 2015-10-27 [PMID: 26501565] (IHC-P, Human)

More publications at <http://www.novusbio.com/NB100-2530>

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





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Products Related to NB100-2530

NB820-59661	Mouse Kidney Whole Tissue Lysate (Adult Whole Normal)
NB100-2530PEP	LOX Antibody Blocking Peptide
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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 100% guarantee, please visit www.novusbio.com/guarantee

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