Product Datasheet

HIF-1 alpha Antibody
NB100-449SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-449SS

HIF-1 alpha Antibody

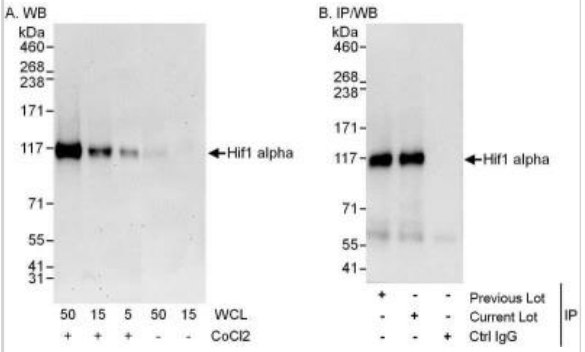
Product Information	
Unit Size	0.025 ml
Concentration	0.2 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-buffered Saline and 0.1% BSA

Product Description	
Host	Rabbit
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Monkey, Primate
Species Reactivity	Monkey (COS-7) and Rat (review feedback). Has been shown to detect HIF-1 alpha in both mouse and human tissue by IHC with Citrate buffer antigen retrieval. Based on 100% sequence identity, this antibody is expected to react with: Cavia porcellus, Panda, Orangutan, Gorilla, Chimpanzee, Grass carp, Northern pike, Atlantic cod, Duckbill platypus and Gansu zokor. Please be aware that this antibody is reactive to Rabbit and derived from the same host, Rabbit. Additional optimization may be required. Please contact Technical Support for more information.
Immunogen	The immunogen recognized by this antibody maps to a region between residues 775 and the C-terminus (residue 826) of human hypoxia-inducible factor 1 (Q16665).

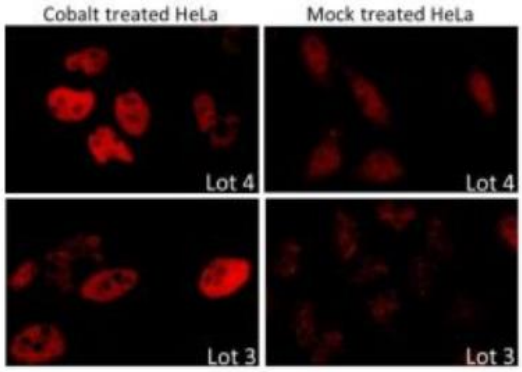
Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay
Recommended Dilutions	Chromatin Immunoprecipitation, ELISA 1:100-1:2000, Flow Cytometry 0.125 ug per 1 million cells in a 150 mcl reaction, Immunocytochemistry/Immunofluorescence 1:10-1:500, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Frozen 1:50-1:200, Immunohistochemistry-Paraffin 1:50-1:200, Immunoprecipitation 2-5 ug/mg lysate, Proximity Ligation Assay 1:200-1:4000, Simple Western 1:200, Western Blot 1:2000-1:10000
Application Notes	This antibody is useful for Western blot, Immunoprecipitation, FLOW, ELISA, Immunocytochemistry/Immunofluorescence and Immunohistochemistry application. For IHC-P, Tris-EDTA pH 9.0 buffer is recommended for the heat induced epitope retrieval.In Simple Western only 10-15 uL of the recommended dilution is used per data point.Use in chromatin immunoprecipitation reported in scientific literature (PMID 25557133)

Images

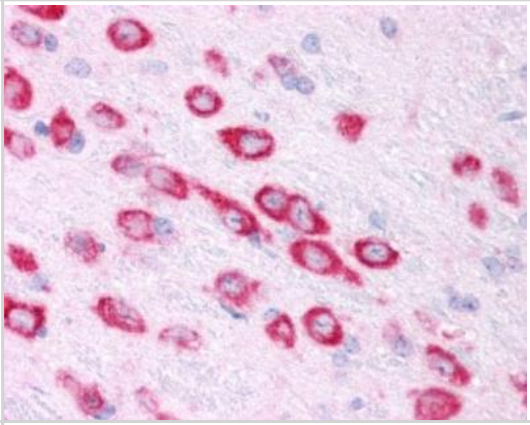
Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of Human HIF1 alpha by Western Blot and Immunoprecipitation. Samples: Whole cell lysate (5, 15 and 50 ug for WB; 1 mg for IP, 20% of IP loaded) from HeLa cells that were either treated with cobalt chloride (+; 200 mcM) or mock treated (-). Antibodies: Affinity purified rabbit anti-HIF1 alpha antibody used for WB at 0.1 ug/ml (A) and 1 ug/ml (B) and used for IP at 3 ug/mg lysate. HIF1 alpha was also immunoprecipitated by a previous lot of this antibody. Detection: Chemiluminescence with exposure times of 30 seconds (A) and 10 seconds (B).



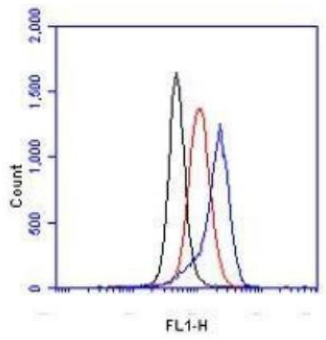
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody [NB100-449] - Formaldehyde-fixed asynchronous HeLa cells.

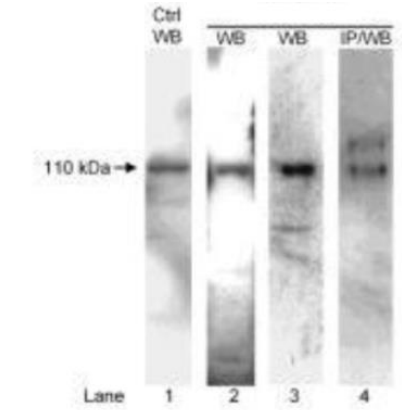
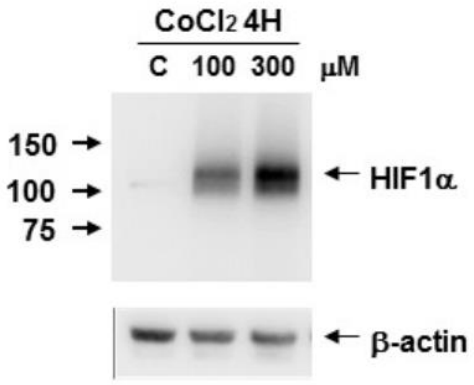
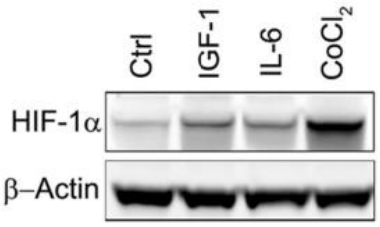
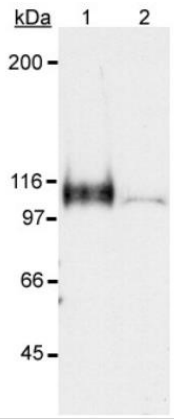


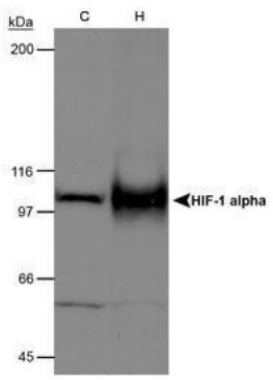
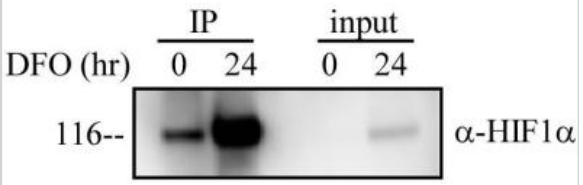
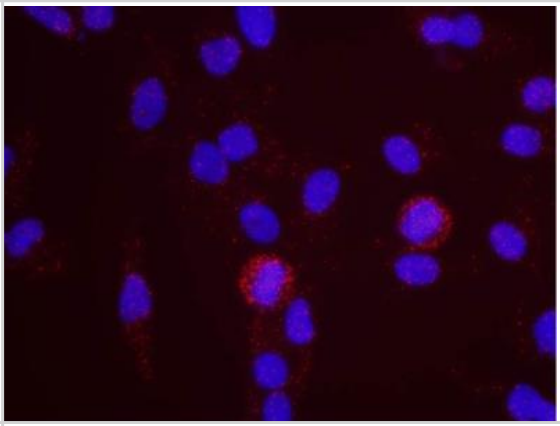
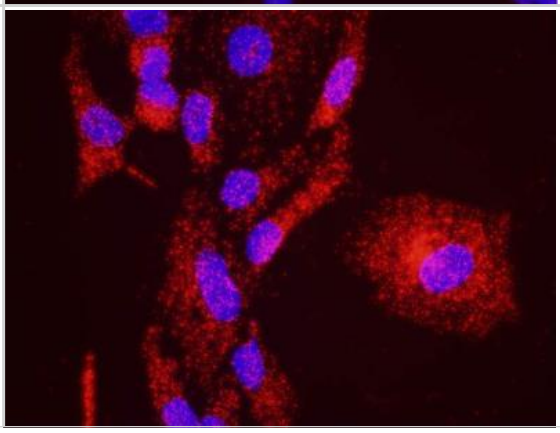
Immunohistochemistry: HIF-1 alpha Antibody [NB100-449] - Mouse Brain, Neurons 40X

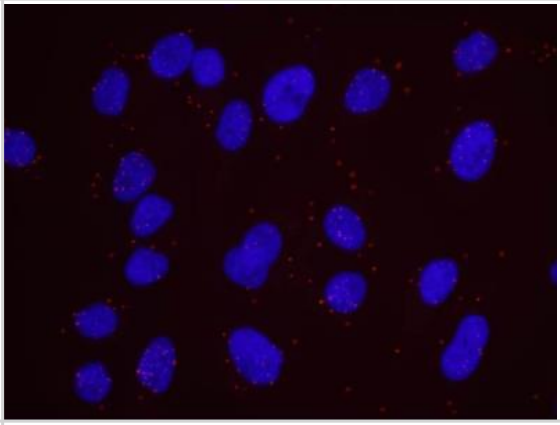
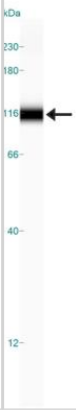


Flow Cytometry: HIF-1 alpha Antibody [NB100-449] - Hela cells were treated for 15 hrs with 200uM CoCl2, fixed in PFA, and permeabilized in 90% MeOH. 1 X 10^6 cells were stained with 0.125ug anti- HIF-alpha and secondary FITC-conjugated goat anti-rabbit (in a 150ul reaction). Black- treated, anti-KLH control IgG; Red- untreated, anti-HIF1-alpha; Blue- treated, anti-HIF1-alpha.



Western Blot: HIF-1 alpha Antibody [NB100-449] - Homogenate from pig (lanes 1 and 2) or rabbit (lane 4) aorta or lysate from cultured rat aortic smooth muscle cells (lane 3). Antibody: Affinity purified rabbit anti-SERCA2 used at 1 ug/ml (lanes 2 and 4) or 0.4 ug/ml (lane 3) for WB and 2 ug/mg lysate for IP or control (ctrl) monoclonal anti-SERCA2 (lanes 1 and 4) used at 1 ug/ml for WB. Detection: Chemiluminescence.	
Western Blot: HIF-1 alpha Antibody [NB100-449] - HIF-1 alpha induction on Caki-1 cell lysate using CoCl2. Image from verified customer review.	
Western Blot: HIF-1 alpha Antibody [NB100-449] - Analysis of HIF-1 alpha in human myeloma cell lysate using anti-HIF-1 alpha. Cells were untreated or treated with IGF-1, IL-6 or CoCl2. Image from verified customer review.	
Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of HIF-1 alpha in a hypoxic sample. Lane 1: CoCl2 treated Cos-7 nuclear extract (hypoxic). Lane 2: Untreated Cos-7 nuclear extract (normoxic).	

Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of mouse HIF1-alpha on hypoxia treated MEFs	
Immunoprecipitation: HIF-1 alpha Antibody [NB100-449] - IP analysis of HIF-1a in HEK293 cells. Image courtesy of anonymous customer review.	
Proximity Ligation Assay: HIF-1 alpha Antibody [NB100-449] - Secondary-conjugate Duolink II PLA in Hela cells. goat anti-human MCM2 (NB100-244) and rabbit anti-human HIF1-alpha (NB100-449). Image merged from DAPI (2ms) and Texas Red (200ms) exposures, 40X magnification.	
Proximity Ligation Assay: HIF-1 alpha Antibody [NB100-449] - Secondary-conjugate Duolink II PLA in Hela cells. goat anti-human MCM7 (NB100-252) and rabbit anti-human HIF1-alpha (NB100-449). Image merged from DAPI (2ms) and Texas Red (200ms) exposures, 40X magnification.	

Proximity Ligation Assay: HIF-1 alpha Antibody [NB100-449] - Secondary-conjugate Duolink II PLA in Hela cells. goat anti-human MCM3 (NB100-249) and rabbit anti-human HIF1-alpha (NB100-449). Image merged from DAPI (2ms) and Texas Red (200ms) exposures, 40X magnification.	
Simple Western: HIF-1 alpha Antibody [NB100-449] - Simple Western lane view shows a specific band for HIF-1 alpha in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Publications

Henique C, Mansouri A, Vavrova E et al. Increasing mitochondrial muscle fatty acid oxidation induces skeletal muscle remodeling toward an oxidative phenotype FASEB J. 2015 Feb 23 [PMID: 25713059] (WB, Mouse)

Teranishi Y, Matsubara T, Krausz KW et al. Involvement of hepatic stellate cell cytoglobin in acute hepatocyte damage through the regulation of CYP2E1-mediated xenobiotic metabolism Lab. Invest. 2015 Feb 16 [PMID: 25686096] (WB, Mouse)

Nusken E, Herrmann Y, Wohlfarth M et al. Strong hypoxia reduces leptin synthesis in purified primary human trophoblasts Placenta. 2015 Feb 03 [PMID: 25684657] (WB, Human)

Chang E, Paterno J, Duscher D et al. Exercise induces stromal cell-derived factor-1alpha-mediated release of endothelial progenitor cells with increased vasculogenic function Plast. Reconstr. Surg. 2015 Feb 01 [PMID: 25626819] (WB, Human)

Matak P, Heinis M, Mathieu JR et al. Myeloid HIF-1 Is Protective in Helicobacter pylori-Mediated Gastritis J. Immunol. 2015 Feb 20 [PMID: 25710915] (IHC-P, Human)

Tsuboi I, Yamashita T, Nagano M et al. Impaired expression of HIF -2alpha induces compensatory expression of HIF-1alpha for the recovery from anemia J. Cell. Physiol. 2015 Jan 03 [PMID: 25557133] (WB, ChIP, Mouse)

Palsson-McDermott EM, Curtis AM, Goel G et al. Pyruvate Kinase M2 Regulates Hif-1alpha Activity and IL-1beta Induction and Is a Critical Determinant of the Warburg Effect in LPS-Activated Macrophages Cell Metab. 2015 Jan 06 [PMID: 25565206] (WB, Mouse)

Kim M, Neinast MD, Frank AP et al. ERalpha upregulates Phd3 to ameliorate HIF-1 induced fibrosis and inflammation in adipose tissue Mol Metab. 2014 Sep 01 [PMID: 25161887] (IP, WB, Mouse)

Details:
HIF-1 alpha antibody used for IP and WB applications in HIF-1 alpha ubiquitination assay on NIH3T3 cells that were transfected with expression vectors for pcDNA-mHIF1a, myc-Ub1, pFlag-ERa and shPHD3 (Figure 2D).

Appel S, Turnwald Em, Ankerne J et al. Hypoxia-Mediated Soluble Fms-Like Tyrosine Kinase 1 Increase is not Attenuated in Interleukin 6-Deficient Mice. Reprod Sci. 2014 Nov 18 [PMID: 25415335]

Kong Ly, Wei J, Haider As et al. Therapeutic targets in subependymoma. J. neuroimmunol. 2014 Oct 31 [PMID: 25465288] (WB, Human)

Kim HR, Kim JH, Choi EJ et al. Hyperoxygenation Attenuated a Murine Model of Atopic Dermatitis through Raising Skin Level of ROS. PLoS ONE. 2014 Oct 03 [PMID: 25275529] (IHC-P, Mouse)

Details:
HIF1 alpha antibody used for IHC-P on the AD lesions from hyperbaric oxygen therapy (HBOT) or applying an oxygen-carrying chemical, perfluorodecalin (PFD) treated murine model of AD that was developed by repeated application of a chemical irritant (1% 2,4-dinitrochlorobenzene) and house dust mite (Dermatophagoide farinae) extract on one ear of BALB/c mice. Figure 6 shows a decreased HIF1 alpha expression in the AD lesions of HBOT or PFD treated mice.

Roychowdhury S, Chiang DJ, McMullen MR et al. Moderate, chronic ethanol feeding exacerbates carbon-tetrachloride-induced hepatic fibrosis via hepatocyte-specific hypoxia inducible factor 1a. Pharmacol Res Perspect 2014 Oct 01 [PMID: 25089199] (IHC-Fr, Mouse)

Details:
HIF1a/HIF-1 alpha antibody used for IHC-Fr on frozen liver sections of pair- or ethanol-fed C57BL/6J wild-type mice (Figure 1B) and hepatocyte-specific HIF1a-deficint mice /delta HepHIF1a-/- on C57BL/6 background fed ethanol or not + CCl4 injections 2X/week for 5 weeks (Figure S1).

More publications at http://www.novusbio.com/NB100-449

Procedures

Western Blot protocol specific for HIF-1 alpha Antibody (NB100-449)
Western Blot Protocol

1. Resolve 5, 15, or 45 ug of HeLa or late passage MEF nuclear cell lysates on an SDS-PAGE.

2. Transfer to either nitrocellulose or PVDF membrane.

3. Block the non-specific protein sites.

4. Incubate the membrane with 0.2 ug/ml of HIF-1 alpha antibody (cat# NB 100-449) at 4C, overnight.
5. Wash the membrane thoroughly.

6. Incubate the membrane with an HRP-conjugated rabbit IgG secondary antibody for 45 min at room temperature.

7. Wash the membrane thoroughly.

8. Use the Supersignal WestPico detection system (Pierce) to develop the membrane.

9. Expose to film for less than 4 seconds.

Western Blot Protocol

1. Perform SDS-PAGE (3-8% Tris-acetate) on samples to be analyzed, loading 50ug of total Cos-7 protein per lane.

2. Transfer proteins to Nitrocellulose membrane according to the instructions provided by the manufacturer of the transfer apparatus.

3. 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 in TBS for 1.5 hours.

6. Dilute the mouse anti-HIF-1 alpha primary antibody (NB 100-449) 1:500 in blocking buffer and incubate overnight at 4C.

7. Wash the membrane in water for 5 min and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1.5 hours at room temperature.

8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.

9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).

10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amershams ECL is the standard reagent used at Novus Biologicals).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunohistochemistry Protocols (NB100-449)

IHC - Frozen 7 um mouse frozen sections were used.
Detection system: Vectors Anti-Rabbit Ig ImmPRESS Reagent Kit (cat # MP-7401)

1. Fix in ice cold acetone

2. Block for one hour at room temp. The block is provided by the vector kit; it is 2.5% horse serum.

3. Use NB 100-449 at a 1:100 dilution in PBS and incubate overnight in the fridge.

4. Perform a 15 min peroxidase block and incubated with the ImmPress anti-rabbit for 30 mins at RT.

5. Use DAB to detect staining and counterstained with Vectors Hemotoxylin. PBS washes (3X2 mins) were done in between all steps except in between the block and the primary

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:

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
A. Place slides in peroxidase quenching solution: 15-30 minutes.

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 90-96C.

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.

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 60C 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 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes)

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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.