Product Datasheet

Fibronectin Antibody - BSA Free
NBP1-91258

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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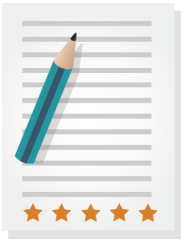
Updated 12/20/2023 v.20.1



NBP1-91258

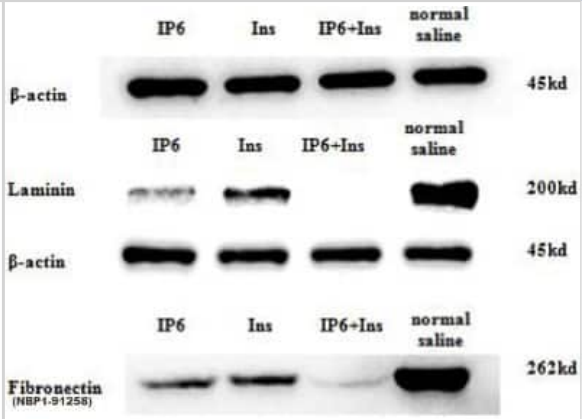
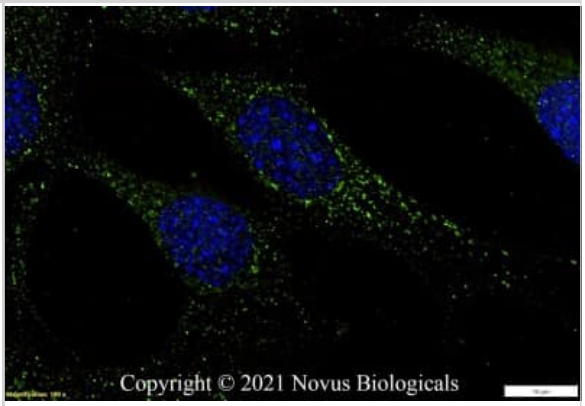
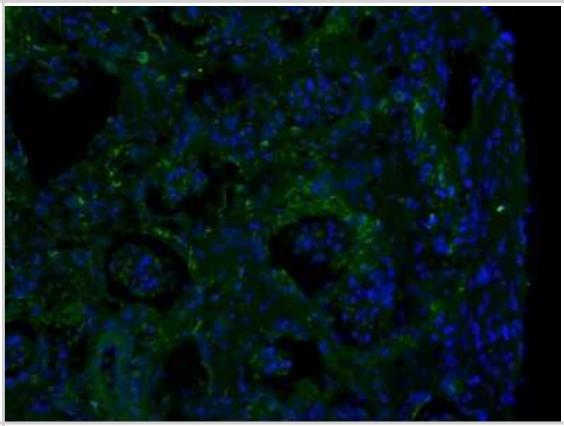
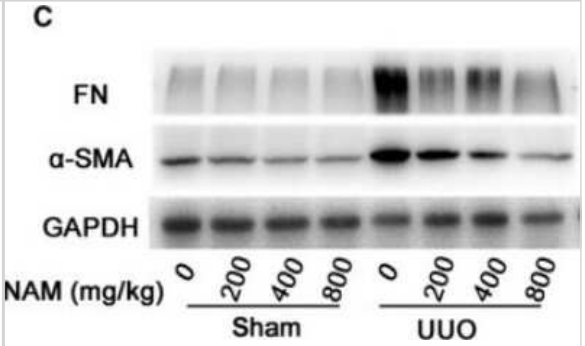
Fibronectin Antibody - BSA Free

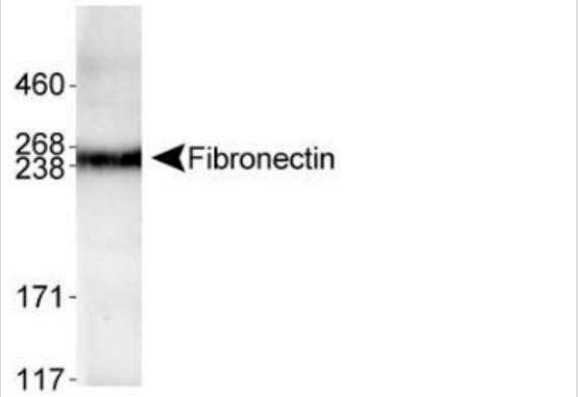
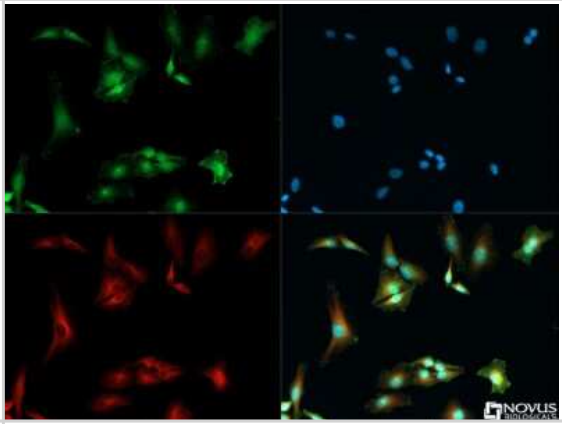
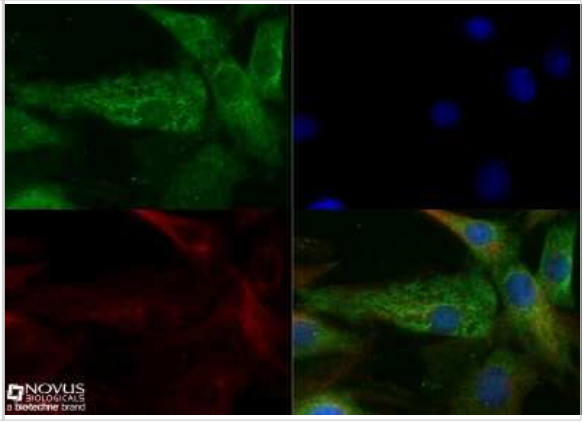
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	2335
Gene Symbol	FN1
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Equine, Feline
Reactivity Notes	Canine reactivity reported in scientific literature (PMID: 29439094). Equine reactivity reported in scientific literature (PMID: 30450188). Rat reactivity reported in scientific literature (PMID: 30699330).
Marker	Mesenchymal Cells Marker
Immunogen	A synthetic peptide made toward the C-terminal region of the human Fibronectin protein (within residues 2250-2300). [Swiss-Prot: P02751]

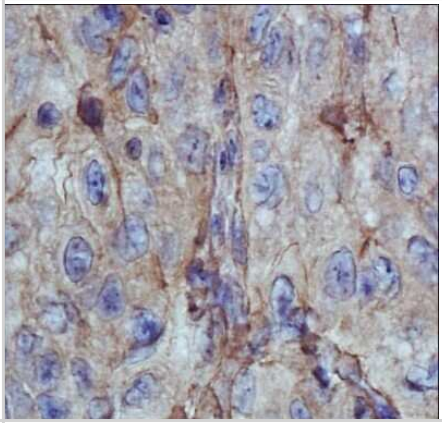
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:100, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:400, Immunohistochemistry-Paraffin 1:400, Immunohistochemistry-Frozen reported by customer review
Application Notes	In Western Blot, a band is seen at ~262 kDa representing Fibronectin. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. In ICC/IF, cytoplasmic staining was observed in HeLa cells. In IHC-P, staining was observed in the cytoplasm and extracellular space of mouse prostate tissue. Antigen retrieval with 10mM sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The 12-230kDa separation system and EZ Standard Pack 5 are recommended for detecting human Fibronectin using Simple Western.

Images

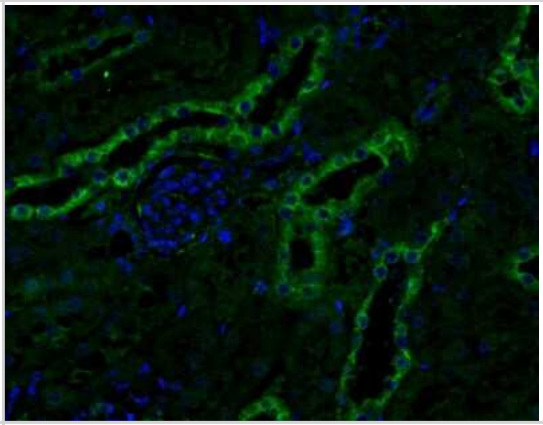
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - VSOP observed in perivascular-restricted spinal cord lesions with intact BBB. Immunostaining for laminin (brown) shows vascular endothelium and glia limitans of a perivascular lesion, along with infiltrating cells and VSOP (blue). Image collected and cropped by CiteAb from the following publication (http://asn.sagepub.com/lookup/doi/10.1042/AN20120081), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: Fibronectin Antibody - BSA Free [NBP1-91258] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NBP1-91258 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis in a 6 month old Alport mouse kidney. Image from a verified customer review.	
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - Nicotinamide (NAM) attenuates unilateral urethral obstruction (UUO)-induced renal interstitial fibrosis. C57BL/6 mice were subjected to UUO surgery or sham operation. Different doses of NAM or saline were intraperitoneally injected an hour before the surgery and daily thereafter. Representative images of Western blot of FN (fibronectin), alpha-SMA (alpha-smooth muscle actin) and GAPDH (loading control). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/30993884/) licensed under a CC-BY license.	

Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in HepG2 cell lysate.	
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in NIH 3T3 cell lysate.	
Immunocytochemistry/Immunofluorescence: Fibronectin Antibody - BSA Free [NBP1-91258] - Fibronectin antibody was tested in HeLa cells with DyLight 488 (Green). Nuclei and alpha-tubulin were counterstained with DAPI (Blue) and DyLight 550 (Red).	
Immunocytochemistry/Immunofluorescence: Fibronectin Antibody - BSA Free [NBP1-91258] - NIH-3T3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Fibronectin at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at 1:500. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at 1:1000 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.	

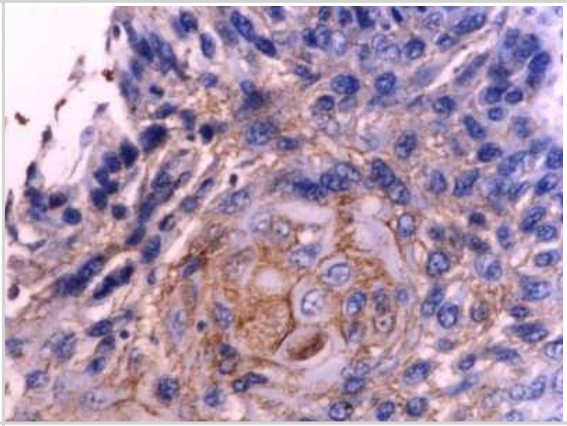
Immunohistochemistry: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in human renal cancer using DAB with hematoxylin counterstain.



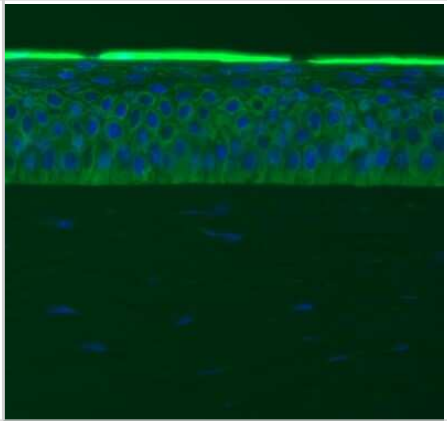
Immunohistochemistry-Paraffin: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in mouse kidney tissue section using anti-Fibronectin antibody. Image from verified customer review.



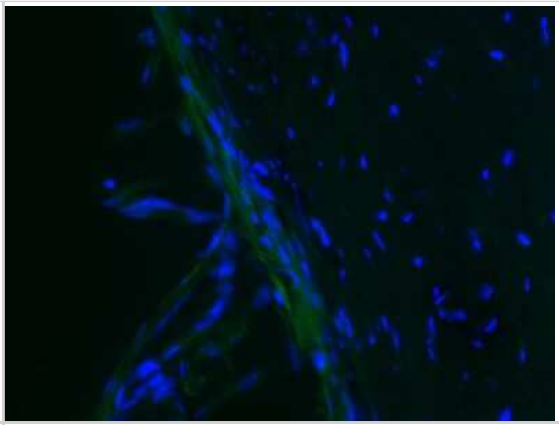
Immunohistochemistry-Paraffin: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in human oesophageal cancer tissue using anti-Fibronectin antibody. Image from verified customer review.



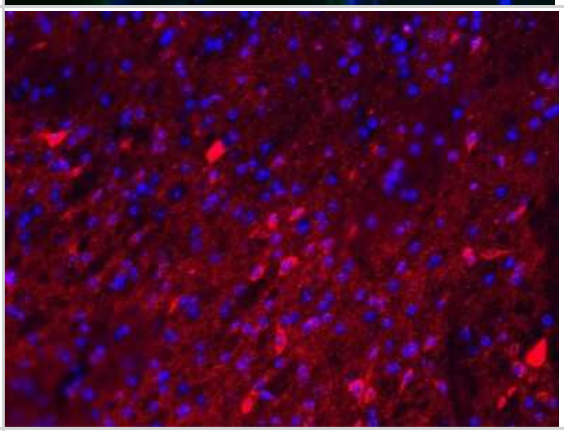
Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Feline corneal stromal cells. IHC-Fr and IF (Alexa Fluor488) were performed using NBP1-91258 at 1:400. Image captured by epifluorescent microscopy. Image from verified customer review.



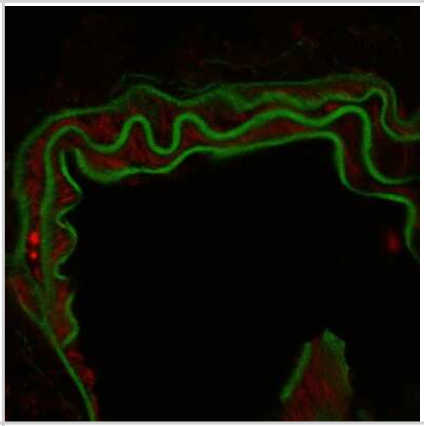
Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Trabecular meshwork (TM) region of pig eyes. NBP1-91258 Fibronectin antibody was labelled with Alexa Fluor 488 conjugated secondary antibody (Green). DAPI shown as blue. Image from verified customer review.



Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Mouse brain cryosections were stained with Fibronectin and anti-rabbit Alexa Fluor 555. Magnification 20x. Image from vrified customer review.

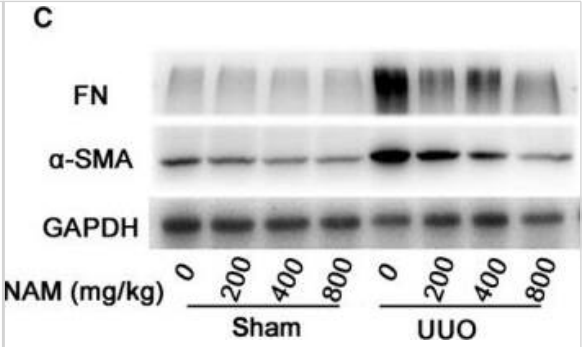


Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Staining in mouse carotid artery. Image from a verified customer review.

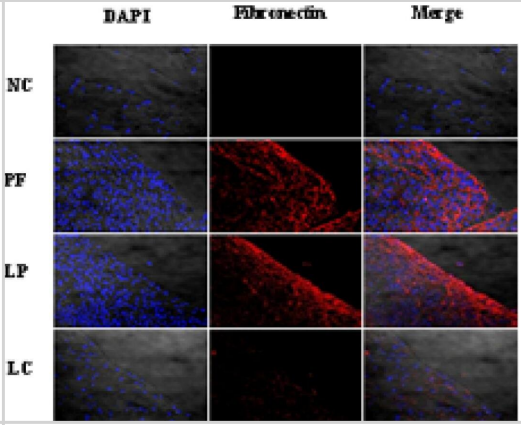


Simple Western: Fibronectin Antibody - BSA Free [NBP1-91258] - Lane view shows a specific band for Fibronectin in 0.5 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

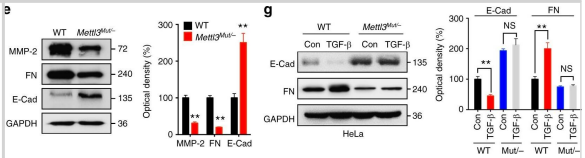
Nicotinamide (NAM) attenuates unilateral urethral obstruction (UUO)-induced renal interstitial fibrosis. C57BL/6 mice were subjected to UUO surgery or sham operation. Different doses of NAM or saline were intraperitoneally injected an hour before the surgery and daily thereafter. The mice were sacrificed at 14 days after surgery to collect obstructed kidneys for histological analysis, Western blot and real-time RT-PCR analysis.(C) Representative images of Western blot of FN (fibronectin), alpha-SMA (alpha-smooth muscle actin) and GAPDH (loading control). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30993884), licensed under a CC-BY licence.



LC depressed both the protein and mRNA level of fibronectin via depletion of peritoneal M2. Values were expressed as the mean +/- SD. (D) Immunofluorescence staining of fibronectin in the four groups. Blue corresponds to nuclear staining, and red corresponds to fibronectin staining. #p < 0.05 vs. NC and LC group. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23685870), licensed under a CC-BY licence.



Lamin A/C-53BP1 interaction is regulated in a DNA damage-dependent manner. (A) Left; HDF were subjected to IR (10 Gy) and allowed to recover for 1 h. Association between A-type lamins and 53BP1 was assessed by immunoprecipitation of endogenous lamin A/C followed by immunoblotting with 53BP1 and lamin A/C antibodies. WCE, whole cell extract, IP: immunoprecipitates. WCE represents 5% input. Right; endogenous lamin A/C was immunoprecipitated and supernatants or pellets were analysed by immunoblotting for interaction with JMJD2A. (B) U2OS/GFP-lamin A cells were pretreated with caffeine (20 mm) for 1 h before exposure to IR (10 Gy, 1 h recovery). Cell extracts were then subjected to immunoprecipitation using GFP-Trap beads, and bound complexes were then analysed by immunoblotting using 53BP1 and GFP antibodies. WCE represents 1% input. (C) As in (C) except cells were pretreated with 10 μm ATMi for 1 h before IR. WCE represents 1% input. (D) U2OS/GFP-lamin A cells were subjected to laser micro-irradiation, fixed 1 h later and immunostained with γ-H2AX antibody. Scale bar, 10 μm. (E) U2OS cells were transfected with siCTRL or siLMNA and subjected to laser micro-irradiation, fixed 15 min later and then processed for immunofluorescence with γ-H2AX and 53BP1 antibodies. Scale bar, 10 μm. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25645366), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Rüegg AB, Kowalewski MP, Ulbrich SE Endometrial extracellular matrix components do not change over the course of embryonic diapause and reactivation in the roe deer (Capreolus capreolus) Reproduction in domestic animals = Zuchthygiene 2023-05-01 [PMID: 36645739] (IHC)

Zhang D, Liu B, Jie X et al. Uncovering Bupi Yishen Formula Pharmacological Mechanisms Against Chronic Kidney Disease by Network Pharmacology and Experimental Validation Frontiers in Pharmacology 2021-11-15 [PMID: 34867380]

Kuczwara V, Schuler G, Pfarrer C et al. Ultrastructural and Immunohistochemical Characterization of Maternal Myofibroblasts in the Bovine Placenta around Parturition Veterinary Sciences 2023-01-07 [PMID: 36669044] (ICC/IF)

Hupy ML, Pedler MG, Shieh B et al. Suppression of epithelial to mesenchymal transition markers in mouse lens by a Smad7-based recombinant protein Chemico-Biological Interactions 2021-08-01 [PMID: 33961834] (EM)

Sen P, Helmke A, Liao CM et al. SerpinB2 Regulates Immune Response in Kidney Injury and Aging Journal of the American Society of Nephrology 2020-05-01 [PMID: 32209589]

Lee GH, Cheon J, Kim D, Jun HS. Lysophosphatidic Acid Promotes Epithelial-Mesenchymal Transition in Kidney Epithelial Cells via the LPAR1/MAPK-AKT/KLF5 Signaling Pathway in Diabetic Nephropathy International Journal of Molecular Sciences 2022-09-10 [PMID: 36142408] (IHC, WB)

Jiang L, Wang YJ, Zhao J et al. Direct Tumor Killing and Immunotherapy through Anti-SerpinB9 Therapy Cell 2020-11-25 [PMID: 33242418]

Sung MS, Kim SY, Eom GH, Park SW High VEGF Concentrations Accelerate Human Trabecular Meshwork Fibrosis in a TAZ-Dependent Manner International journal of molecular sciences 2023-06-01 [PMID: 37298577] (WB, Human)

Wu X, Zhang D, Qiao X et al. Regulating the cell shift of endothelial cell-like myofibroblasts in pulmonary fibrosis The European respiratory journal 2023-06-01 [PMID: 36758986] (WB, Mouse)

Passanha FR, Geuens T, LaPointe VLS Cadherin-11 Influences Differentiation in Human Mesenchymal Stem Cells by Regulating the Extracellular Matrix Via the TGF?1 Pathway Stem cells (Dayton, Ohio) 2022-07-27 [PMID: 35416252] (WB, ICC/IF, Human)

Li XF, Selli C, Zhou HL et al. Macrophages promote anti-androgen resistance in prostate cancer bone disease The Journal of experimental medicine 2023-04-03 [PMID: 36749798] (IHC-P, ICC/IF, Mouse)

Details:
Dilution used in IHC-P and ICC 1:500

Barreto R, Carvalho H, Matias G et al. THE EXTRACELLULAR MATRIX PROTEIN PATTERN IN THE CANINE NEOPLASTIC MAMMARY GLAND Tissue and Cell 2023-02-01 [PMID: 36933273] (IHC, Canine)

More publications at http://www.novusbio.com/NBP1-91258

Procedures

Western Blot protocol for Fibronectin Antibody (NBP1-91258)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry-Paraffin protocol for Fibronectin Antibody (NBP1-91258)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Immunocytochemistry/Immunofluorescence protocol for Fibronectin Antibody (NBP1-91258)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

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2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
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Products Related to NBP1-91258

NBP1-42569	HepG2 Whole Cell Lysate
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.

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