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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Reviews: 11 Publications: 56

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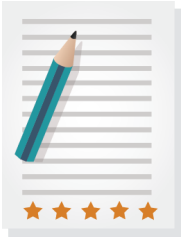
Updated 9/9/2025 v.20.1



NBP1-91258

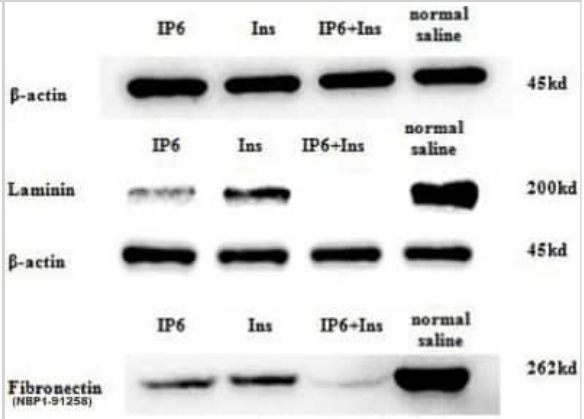
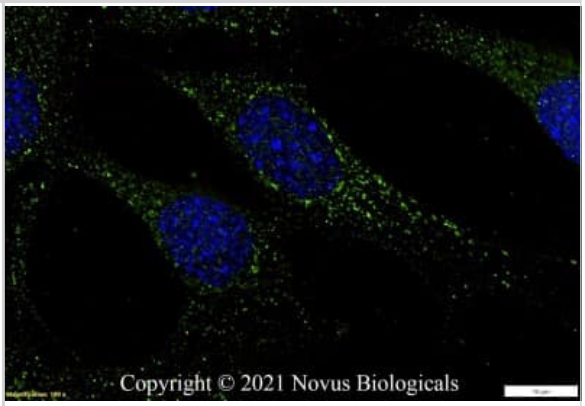
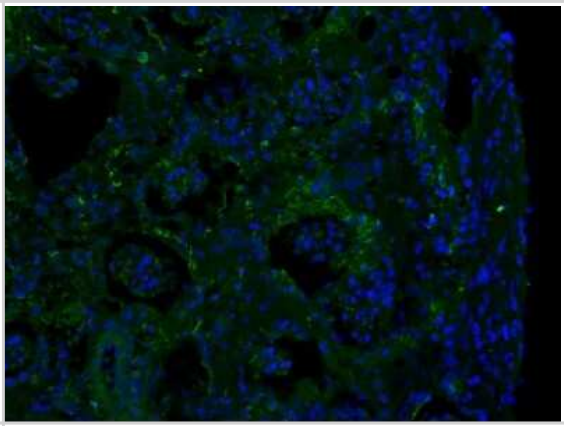
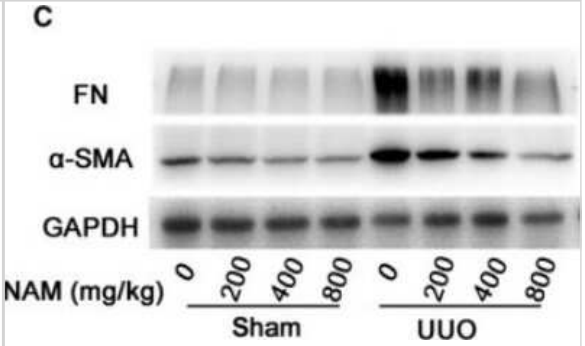
Fibronectin Antibody - BSA Free

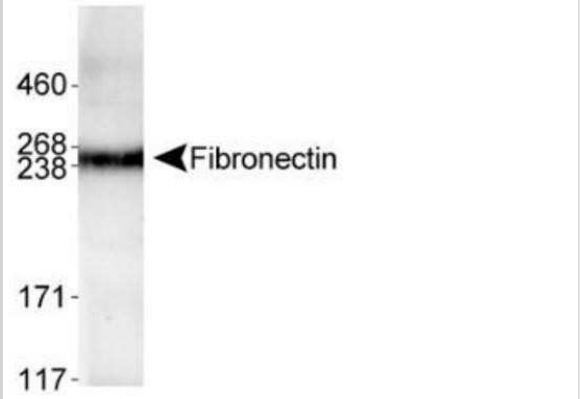
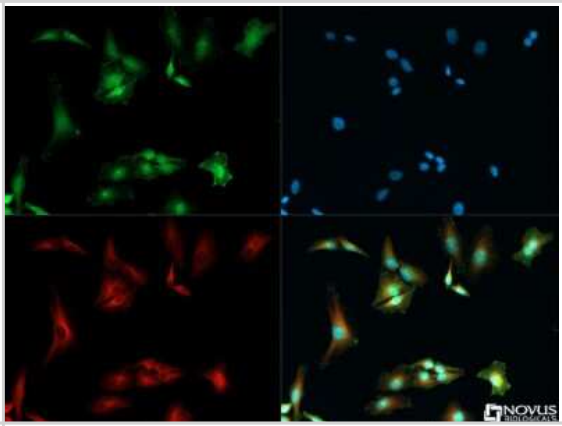
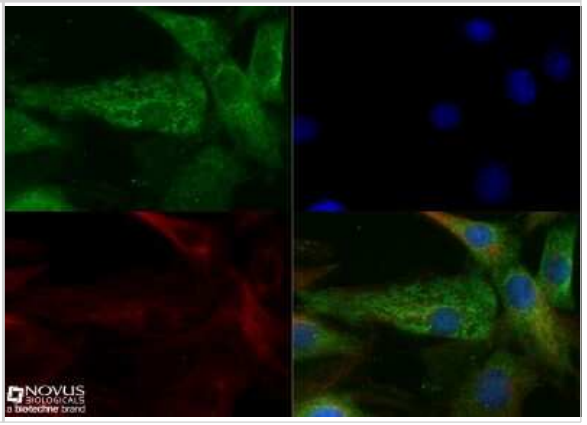
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit Fibronectin Antibody - BSA Free (NBP1-91258) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and Simple Western. Anti-Fibronectin Antibody: Cited in 56 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
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 (canonical isoform within residues 2300-2380). [Swiss-Prot: P02751]

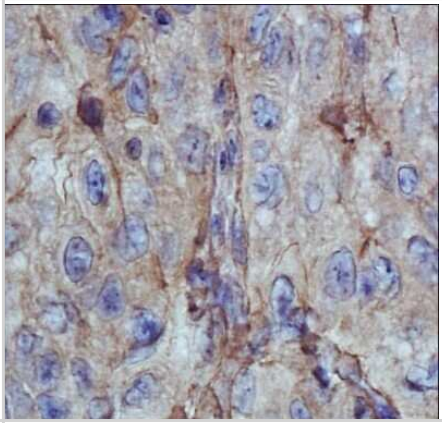
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:100, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1 ug/ml, Immunohistochemistry-Paraffin 1:200, 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. See Simple Western Antibody Database for Simple Western validation: Tested in HepG2 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100. 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

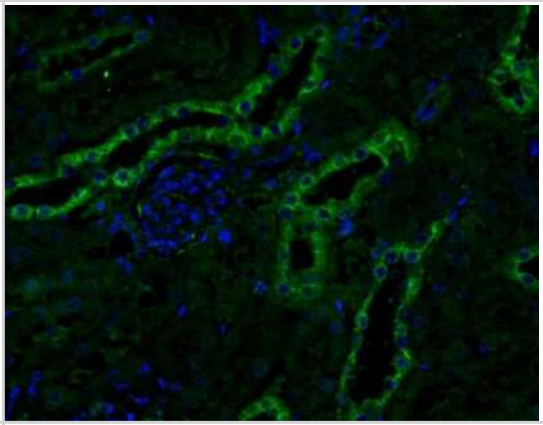
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - VSOP observed in perivascular-restricted spinal cord lesions with intact BBB. Immunostaining for laminin (brown) shows vascular endothelium and glia limitans of a perivascular lesion, along with infiltrating cells and VSOP (blue). Image collected and cropped by CiteAb from the following publication (https://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.	

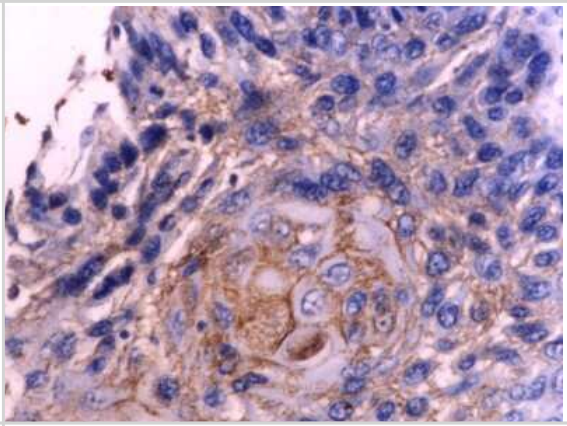
Immunohistochemistry: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in human renal cancer using DAB with hematoxylin counterstain.



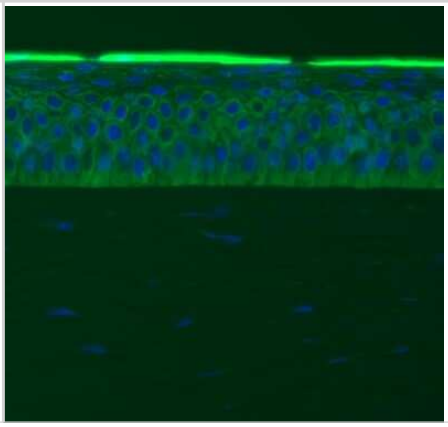
Immunohistochemistry-Paraffin: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in mouse kidney tissue section using anti-Fibronectin antibody. Image from verified customer review.



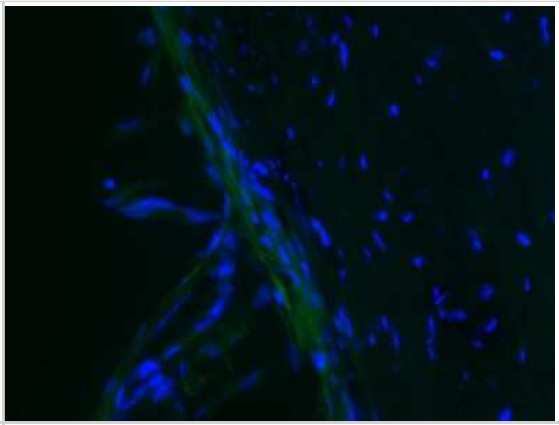
Immunohistochemistry-Paraffin: Fibronectin Antibody - BSA Free [NBP1-91258] - Analysis of Fibronectin in human oesophageal cancer tissue using anti-Fibronectin antibody. Image from verified customer review.



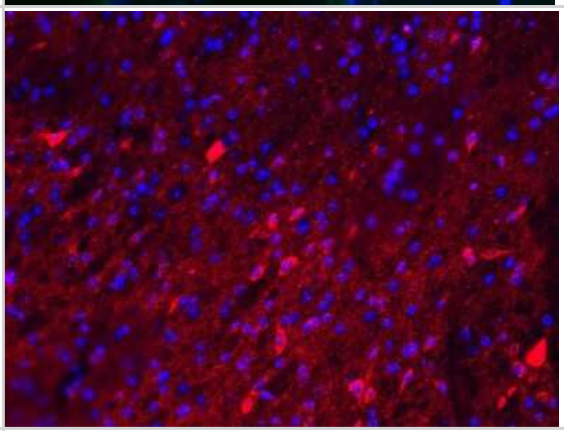
Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Feline corneal stromal cells. IHC-Fr and IF (Alexa Fluor488) were performed using NBP1-91258 at 1:400. Image captured by epifluorescent microscopy. Image from verified customer review.



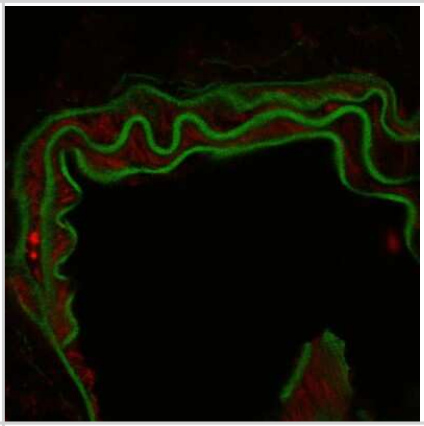
Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Trabecular meshwork (TM) region of pig eyes. NBP1-91258 Fibronectin antibody was labelled with Alexa Fluor 488 conjugated secondary antibody (Green). DAPI shown as blue. Image from verified customer review.



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.

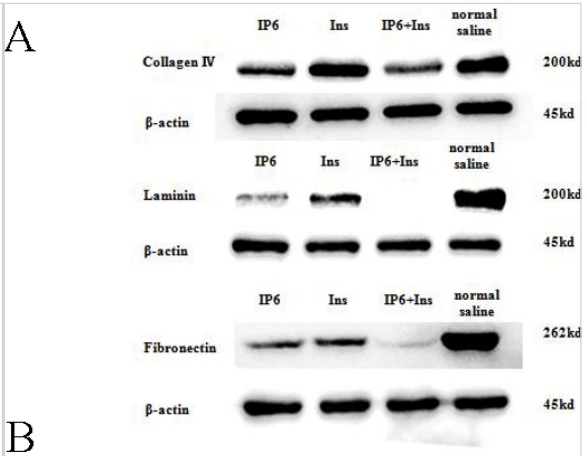


Immunohistochemistry-Frozen: Fibronectin Antibody - BSA Free [NBP1-91258] - Staining in mouse carotid artery. Image from a verified customer review.

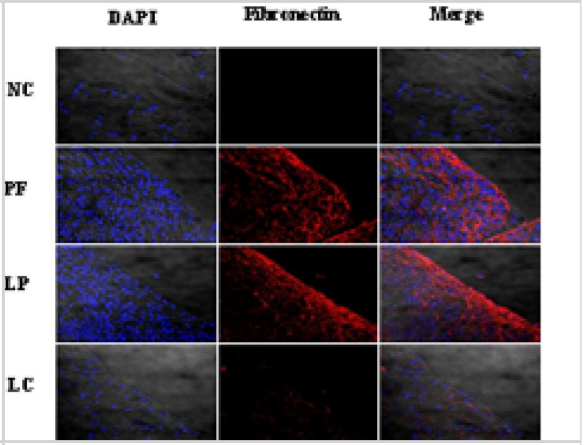


Simple Western: Fibronectin Antibody - BSA Free [NBP1-91258] - Lane view shows a specific band for Fibronectin in 0.5 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

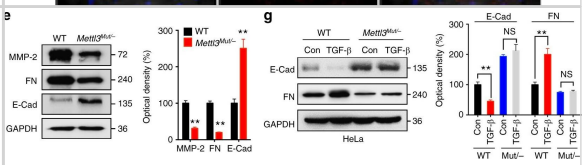
Western blot analysis of the effects of IP6, Ins, IP6 + Ins & normal saline on the levels of collagen IV, Lamininand Fibronectin. IP6 or Ins treatment decreased the protein expression of collagen IV, LN & FN, & the combined IP6 + Ins treatment resulted in significantly greater effects compared with treatment with either compound alone. The samples were probed with antibodies against p-collagen IV, p-LN, & p-FN. The Western blot membranes were stripped & reprobed for β-actin as an internal control to confirm equal loading. (A) representative blots from one of three separate experiments; (B) relative band intensities based on densitometry. The results are expressed as the mean ± standard deviation from three independent experiments. * $p < 0.05$ compared to the IP6 + Ins group; #$p < 0.05$ compared to the normal saline group. Image collected & cropped by CiteAb from the following publication (http://www.mdpi.com/2072-6643/8/5/286), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



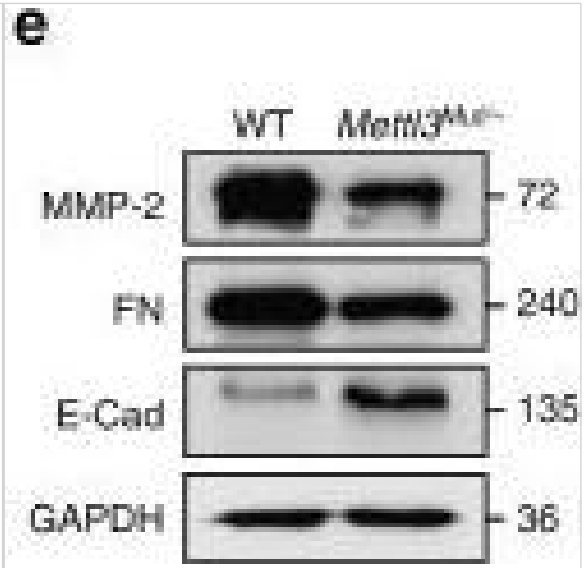
Immunocytochemistry/ Immunofluorescence: Fibronectin Antibody - BSA Free [NBP1-91258] - LC depressed both the protein & mRNA level of fibronectin via depletion of peritoneal M2. Values were expressed as the mean ± SD. (A) The overexpression of fibronectin induced by Lactate-4.25% dialysate was evidently downregulated by LC treatment measured by Western blotting; (B) The relative protein level of fibronectin normalized by GAPDH; (C) The mRNA level of fibronectin was depressed by LC treatment; (D) Immunofluorescence staining of fibronectin in the four groups. Blue corresponds to nuclear staining, & red corresponds to fibronectin staining. #$p < 0.05$ vs. NC & LC group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23685870), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



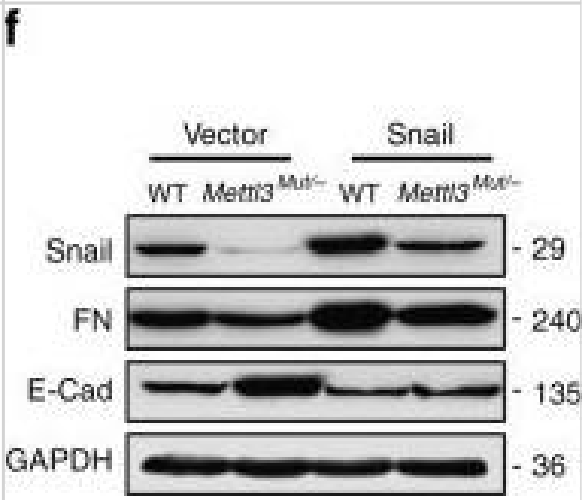
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - EMT in cancer cells is regulated by m6A levels of mRNAs. a HeLa & HepG2 cells were treated with or without 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. b Wound healing of wild-type (control) or Mettl3Mut/− cells was recorded (left) & quantitatively analyzed (right). c Wild-type or Mettl3Mut/− cells were allowed to invade for 24 h & tested by CytoSelect™ 24-well Cell Invasion assay kits (8 μm, colorimetric format); d, e mRNA (d) & protein (e) expressions of MMP2, FN, & E-Cad in wild-type & Mettl3Mut/− HeLa cells were measured by qRT-PCR & western blot analysis, respectively. f HeLa cells were transfected with pcDNA/ALKBH5 or a vector control for 48 h, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). g Wild-type or Mettl3Mut/− cells were treated with or without 10 ng/ml TGF-β for 3 days, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). h The expression of METTL3 in liver cancer & its matched adjacent normal tissues of 50 patients from TCGA database. i Correlation between METTL3 & CDH1 in liver cancer patients (n = 364) from TCGA database. j HeLa cells were pretreated with or without Smad2/3 inhibitor SB431542 (10 μM) & then further treated with 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. Data are presented as means ± SD from three independent experiments. *$p < 0.05$, **$p < 0.01$, NS, no significant, by Student's t test. Red bar = 200 μm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31061416), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



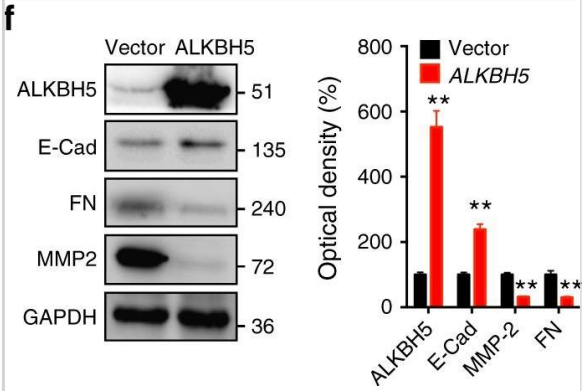
Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - EMT in cancer cells is regulated by m6A levels of mRNAs. a HeLa & HepG2 cells were treated with or without 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. b Wound healing of wild-type (control) or Mettl3Mut/− cells was recorded (left) & quantitatively analyzed (right). c Wild-type or Mettl3Mut/− cells were allowed to invade for 24 h & tested by CytoSelect™ 24-well Cell Invasion assay kits (8 µm, colorimetric format); d, e mRNA (d) & protein (e) expressions of MMP2, FN, & E-Cad in wild-type & Mettl3Mut/− HeLa cells were measured by qRT-PCR & western blot analysis, respectively. f HeLa cells were transfected with pcDNA/ALKBH5 or a vector control for 48 h, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). g Wild-type or Mettl3Mut/− cells were treated with or without 10 ng/ml TGF-β for 3 days, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). h The expression of METTL3 in liver cancer & its matched adjacent normal tissues of 50 patients from TCGA database. i Correlation between METTL3 & CDH1 in liver cancer patients (n = 364) from TCGA database. j HeLa cells were pretreated with or without Smad2/3 inhibitor SB431542 (10 µM) & then further treated with 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. Data are presented as means ± SD from three independent experiments. *p < 0.05, **p < 0.01, NS, no significant, by Student's t test. Red bar = 200 µm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31061416), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - Snail is involved in m6A-regulated EMT in cancer cells. a Overlapping of 2.0-fold m6A expression changes in EMT cells & EMT-related functional genes. b m6A peaks were enriched in CDS & 3′UTRs of SNAI1 genes from m6A RIP-seq data. Squares marked increases of m6A peaks in cancer cells undergoing EMT; c m6A RIP-qPCR analysis of SNAI1 mRNA in the control & EMT undergoing HeLa cells. d Protein expression of Snail in Mettl3Mut/− or AKLBH5 transfected (24 h) HeLa cells & the control. e The wound healing of wild-type or Mettl3Mut/− HeLa cells transfected with or without pcDNA/Snail for 48 h were recorded (left) & quantitatively analyzed (right). f Wild-type or Mettl3Mut/− HeLa cells were transfected with or without pcDNA/Snail for 48 h, expression of Snail, FN & E-Cad were measured by western blot analysis. Data are presented as means ± SD from three independent experiments. *p < 0.05, NS, no significant, by Student's t test. Red bar = 200µm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31061416), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

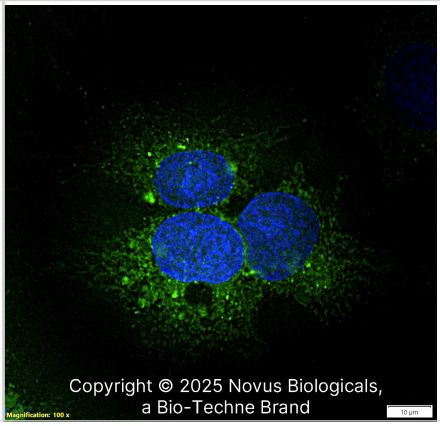


Western Blot: Fibronectin Antibody - BSA Free [NBP1-91258] - EMT in cancer cells is regulated by m6A levels of mRNAs. a HeLa & HepG2 cells were treated with or without 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. b Wound healing of wild-type (control) or Mettl3Mut/− cells was recorded (left) & quantitatively analyzed (right). c Wild-type or Mettl3Mut/− cells were allowed to invade for 24 h & tested by CytoSelect™ 24-well Cell Invasion assay kits (8 μm, colorimetric format); d, e mRNA (d) & protein (e) expressions of MMP2, FN, & E-Cad in wild-type & Mettl3Mut/− HeLa cells were measured by qRT-PCR & western blot analysis, respectively. f HeLa cells were transfected with pcDNA/ALKBH5 or a vector control for 48 h, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). g Wild-type or Mettl3Mut/− cells were treated with or without 10 ng/ml TGF-β for 3 days, protein expression was determined by western blot analysis (left) & quantitatively analyzed (right). h The expression of METTL3 in liver cancer & its matched adjacent normal tissues of 50 patients from TCGA database. i Correlation between METTL3 & CDH1 in liver cancer patients (n = 364) from TCGA database. j HeLa cells were pretreated with or without Smad2/3 inhibitor SB431542 (10 μM) & then further treated with 10 ng/ml TGF-β for 3 days, the m6A/A ratio of the total mRNA were determined by LC–MS/MS. Data are presented as means ± SD from three independent experiments. *p < 0.05, **p < 0.01, NS, no significant, by Student's t test. Red bar = 200 μm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31061416), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Fibronectin was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-Fibronectin Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-91258AF647) (light blue) at 10 μg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.

Fibronectin was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-Fibronectin Affinity Purified Polyclonal Antibody conjugated to FITC (Catalog # NBP1-91258F) (green) at 10 μg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

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] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

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] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

Jiang L, Wang YJ, Zhao J et al. Direct Tumor Killing and Immunotherapy through Anti-SerpinB9 Therapy Cell 2020-11-25 [PMID: 33242418] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

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] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

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] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

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] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

S Dey, LM Udari, P RiveraHern, JJ Kwon, B Willis, JJ Easler, EL Fogel, S Pandol, J Kota Loss of miR-29a/b1 promotes inflammation and fibrosis in acute pancreatitis JCI Insight, 2021-10-08;0(0):. 2021-10-08 [PMID: 34464354] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

Schmitt C, Lechanteur A, Cossais F et al. Liposomal Encapsulated Curcumin Effectively Attenuates Neuroinflammatory and Reactive Astrogliosis Reactions in Glia Cells and Organotypic Brain Slices International Journal of Nanomedicine 2020-05-25 [PMID: 32547020] (Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Mouse)

Ana C. Acosta, Hadi Joud, Mei Sun, Marcel Y. Avila, Curtis E. Margo, Edgar M. Espana Keratocyte-Derived Myofibroblasts: Functional Differences With Their Fibroblast Precursors Investigative Ophthalmology & Visual Science 2023-10-05 [PMID: 37796488]

Nan Zhang, Pengyu Zhang, Xizhi Deng, Min Zhu, Yixin Hu, Dongxiao Ji, Lufan Li, Yang Liu, Wen Zeng, Min Ke Protective Effect of Nicotinamide Riboside on Glucocorticoid-Induced Glaucoma: Mitigating Mitochondrial Damage and Extracellular Matrix Deposition Investigative Ophthalmology & Visual Science 2024-07-01 [PMID: 38949632]

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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.

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

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

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.

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