

Product Datasheet

Fibronectin Antibody 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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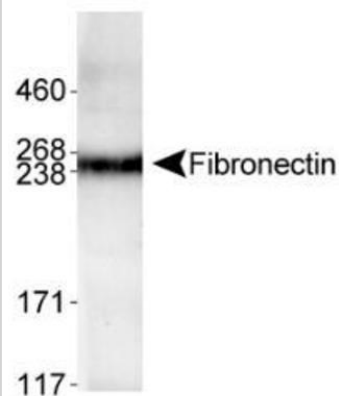
NBP1-91258

Fibronectin Antibody

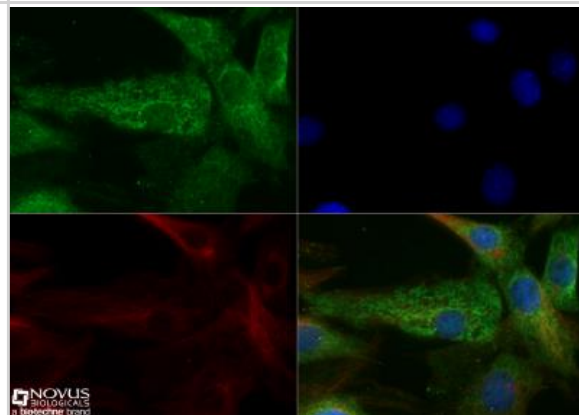
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol
Product Description	
Host	Rabbit
Gene ID	2335
Gene Symbol	FN1
Species	Human, Mouse, Bovine, Canine, Feline
Reactivity Notes	Human and Mouse. Canine reactivity reported in scientific literature (PMID: 29439094). Based on immunogen's sequence homology, this antibody is predicted to react with Chicken (91%), Rat (95%), and Equine/Horse (100%). Immunogen similarity with other species: Xenopus (86%) and Newt (82%).
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 1:40, Immunohistochemistry-Paraffin 1:400, Immunohistochemistry-Frozen
Application Notes	In ICC/IF, cytoplasmic staining was observed in HeLa cells. In Western Blot, a band is seen ~262 kDa representing Fibronectin. In IHC-P, staining was observed in the cytoplasm and extracellular space of mouse prostate tissue. In IHC-P, 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.

Images

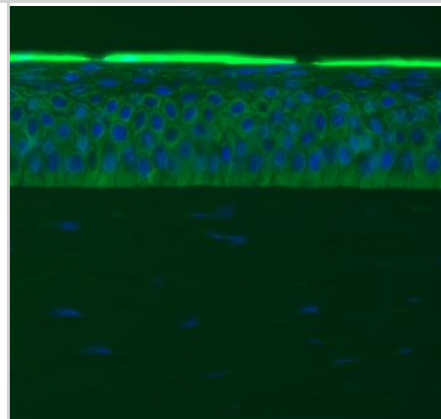
Western Blot: Fibronectin Antibody [NBP1-91258] - WB analysis of Fibronectin in NIH 3T3 cell lysate.



Immunocytochemistry/Immunofluorescence: Fibronectin Antibody [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-X100. The cells were incubated with anti-Fibronectin at 2 ug/ml 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.



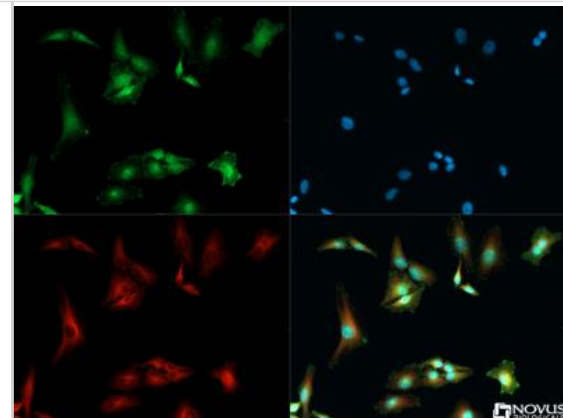
Immunohistochemistry-Frozen: Fibronectin Antibody [NBP1-91258] - Feline corneal stromal cells. IHC-FR and IF (Alexa Fluor488) was performed using NBP1-91258 at 1:400 dilution. Image was captured by epifluorescent microscopy. Image from verified customer review.



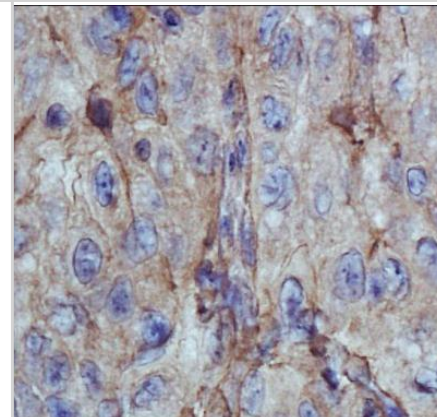
Western Blot: Fibronectin Antibody [NBP1-91258] - WB analysis of Fibronectin in HepG2 cell lysate.



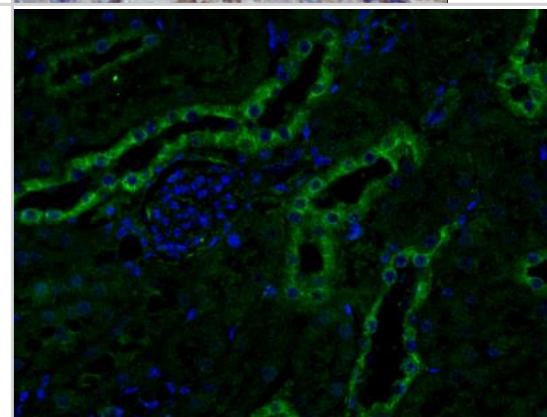
Immunocytochemistry/Immunofluorescence: Fibronectin Antibody [NBP1-91258] - Fibronectin antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



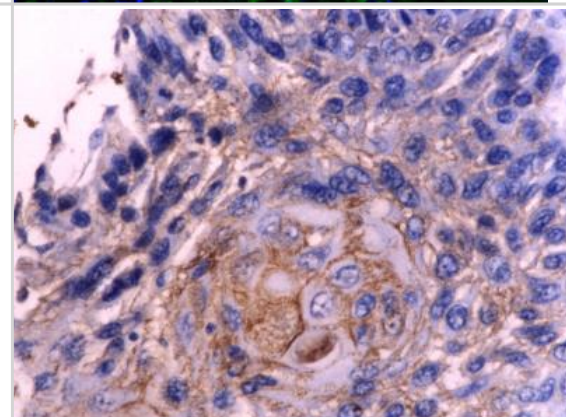
Immunohistochemistry: Fibronectin Antibody [NBP1-91258] - IHC analysis of Fibronectin in human renal cancer using DAB with hematoxylin counterstain.



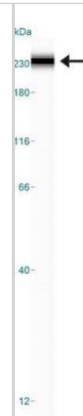
Immunohistochemistry-Paraffin: Fibronectin/Anastellin Antibody [NBP1-91258] - analysis of Fibronectin in mouse kidney tissue section using anti-Fibronectin antibody. Image from verified customer review.



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



Simple Western: Fibronectin/Anastellin Antibody [NBP1-91258] - Simple Western 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.



Publications

Matias GSS, Rigoglio NN, Carreira ACO et al. Optimization of Canine Placenta Decellularization: An Alternative Source of Biological Scaffolds for Regenerative Medicine. *Cells Tissues Organs (Print)* Sep 10 2018 12:00AM [PMID: 30199873] (IHC-P, Canine)

Nitta Kyoko, Shi Sen, Nagai Takako et al. Oral Administration of N-Acetyl-seryl-aspartyl-lysyl-proline Ameliorates Kidney Disease in Both Type 1 and Type 2 Diabetic Mice via a Therapeutic Regimen. *BioMed Research International* 2016 [PMID: 27088094] (WB, Mouse)

Graubner FR, Boos A, Aslan S, Kucukaslan I. Uterine and placental distribution of selected extracellular matrix (ECM) components in the dog. *Reproduction*. 2018 Feb 09 [PMID: 29439094] (Canine)

Jung MY, Kang JH, Hernandez DM, Yin X. Fatty acid synthase is required for profibrotic TGF- β signaling. *FASEB J*. 2018 Feb 16 [PMID: 29475397] (IHC, Mouse)

da Silva Nunes Barreto R, Romagnolli P, Mess AM, Miglino MA. Decellularized bovine cotyledons may serve as biological scaffolds with preserved vascular arrangement *J Tissue Eng Regen Med*. 2017 Nov 21 [PMID: 29164819] (Bovine)

Chiang KC, Yeh CN, Pang JS et al. 1 α ,25(OH) $_2$ D $_3$ Analog, MART-10, Inhibits Neuroendocrine Tumor Cell Metastasis After VEGF-A Stimulation. *Anticancer Res*. 2017 Nov 01 [PMID: 29061804] (Human)

Fu M, Song Y, Wen Z et al. Inositol Hexaphosphate and Inositol Inhibit Colorectal Cancer Metastasis to the Liver in BALB/c Mice. *Nutrients* [PMID: 27187454]

Green CJ, Fraser ST, Day ML. Insulin-like growth factor 1 increases apical fibronectin in blastocysts to increase blastocyst attachment to endometrial epithelial cells in vitro. *Hum. Reprod*. 2015 Feb 01 [PMID: 25432925] (ICC/IF, Mouse)

Details:

Fibronectin/Anastellin antibody used for ICC-IF on Day 5 blastocysts that were cultured in serum starved medium for 24, 48 or 72 h in the absence or presence of IGF1 or in the presence of a PI3K inhibitor LY294002 or in the presence of IGF1, attached to a coverslip - 15 minute 4% paraformaldehyde fixation, 30 minutes permeabilization with PBS-Polyvinyl alcohol-0.3% Triton X100, blocking in PPTB (PBS + PVA + 0.1% Tween-20 + 0.7% BSA), ON 4C incubation of primary, anti-rabbit Alexa 488 secondary antibody (Figure 5).

Medina-Ortiz WE, Belmares R, Neubauer S et al. Cellular Fibronectin Expression in Human Trabecular Meshwork and Induction by Transforming Growth Factor- β 2. *Invest Ophthalmol Vis Sci*. 2013 Oct 17 [PMID: 24030464]

Wang J, Jiang ZP, Su N et al. The role of peritoneal alternatively activated macrophages in the process of peritoneal fibrosis related to peritoneal dialysis. *Int J Mol Sci* 2013 May 17 [PMID: 23685870] (IHC-P, ICC/IF, Mouse)

Hosick PA, Alamodi AA, Storm MV et al. Chronic carbon monoxide treatment attenuates development of obesity and remodels adipocytes in mice fed a high-fat diet. *Int J Obes (Lond)* 2013 Apr 30 [PMID: 23689359] (WB, Mouse)

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 1:25,000 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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NBP1-42569	HepG2 Whole Cell Lysate
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NB7156	Goat anti-Rabbit IgG (H+L) Secondary Antibody
NBP2-24891	Rabbit IgG Isotype Control

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