Product Datasheet

Fibronectin Antibody
NBP1-91258

Unit Size: 0.1 ml

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

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Updated 10/25/2018 v.20.1

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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.
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.

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).


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


Chiang KC, Yeh CN, Pang JS et al. 1α,25(OH)2D3 Analog, MART-10, Inhibits Neuroendocrine Tumor Cell Metastasis After VEGF-A Stimulation. Anticancer Res. 2017 Nov 01 [PMID: 29061804] (Human)


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).


**Procedures**

**Western Blot protocol for Fibronectin Antibody (NBP1-91258)**

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 μg 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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2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
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**Limitations**
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