# **Product Datasheet**

# Osteopontin/OPN Antibody (1B20) - BSA Free NB110-89062

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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## NB110-89062

Osteopontin/OPN Antibody (1B20) - BSA Free

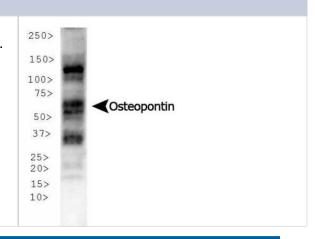
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Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1B20
Preservative	0.05% Sodium Azide
Isotype	lgG1
Purity	Protein G purified
Buffer	PBS
Product Description	

Product Description	
Description	Novus Biologicals Mouse Osteopontin/OPN Antibody (1B20) - BSA Free (NB110 -89062) is a monoclonal antibody validated for use in IHC, WB, ICC/IF, Simple Western and IP. Anti-Osteopontin/OPN Antibody: Cited in 34 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	6696
Gene Symbol	SPP1
Species	Human, Mouse, Rat, Rabbit
Reactivity Notes	Human, Rat and Rabbit. Rabbit reactivity reported in scientific literature (PMID:33001866). Use in Mouse reported in scientific literature (PMID:33580126).
Immunogen	Synthetic peptide corresponding to the C-terminus of human Osteopontin [Swiss-Prot# P10451].

<b>Product Application Details</b>	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:20, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen

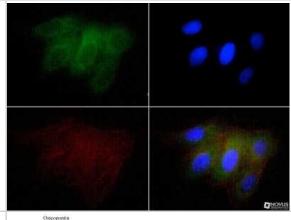
# **Images**

Western Blot: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Analysis of Osteopontin expression in U2OS whole cell lysate.





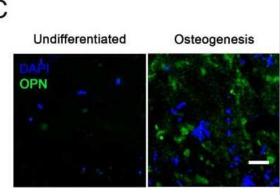
Immunocytochemistry/Immunofluorescence: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Osteopontin antibody was tested at 1:50 in U2OS cells with FITC (green). Nuclei and actin were counterstained with Dapi (blue) and Phalloidin (red).



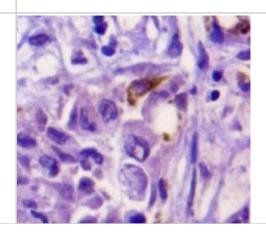
Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Immunohistological results at 12 weeks for (A) Group I, (B) Group II (C) Group III. The pictures are arranged by staining technique (columns) and by the investigated treatment (rows). Areas that stained positive for osteopontin are indicated by red arrowheads. Image collected and cropped by CiteAb from the following publication (https://peerj.com/articles/3513) licensed under a CC-BY license.



Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Osteogenic differentiation in 3D. Immunostaining for osteopontin (OPN) and nuclear DAPI in 3D cultures after 5 weeks. Scale bar = 50 um. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/34948393/) licensed under a CC-BY license.



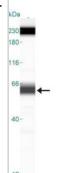
Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Analysis of Osteopontin on human lung adenocarcinoma.



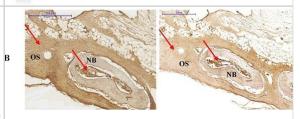
Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Osteopontin in (A) Group I, (B) Group II (C) Group III at 6 weeks. The pictures are arranged by staining technique (columns) and the investigated treatment (rows). Areas that stained positive for osteocalcin and osteopontin are indicated by red arrowheads. NB, new bone; OS, osteoid; FM, fatty marrow. Image collected and cropped by CiteAb from the following publication (https://peerj.com/articles/3513) licensed under a CC-BY license.



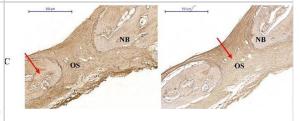
Simple Western: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Simple Western lane view shows a specific band for Osteopontin in 0.5 mg/ml of Human Breast Cancer lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. \* Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody



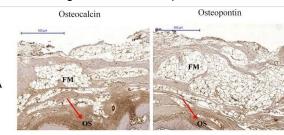
Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Photomicrographs of immunostaining for osteocalcin & osteopontin in (A) Group I, (B) Group II (C) Group III at 6 weeks. The pictures are arranged by staining technique (columns) & the investigated treatment (rows). Areas that stained positive for osteocalcin & osteopontin are indicated by red arrowheads. NB, new bone; OS, osteoid; FM, fatty marrow. Image collected & cropped by CiteAb from the following publication (https://peerj.com/articles/3513), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



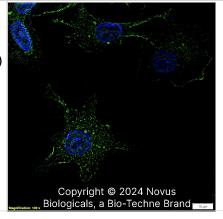
Immunohistochemistry: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Photomicrographs of immunostaining for osteocalcin & osteopontin in (A) Group I, (B) Group II (C) Group III at 6 weeks. The pictures are arranged by staining technique (columns) & the investigated treatment (rows). Areas that stained positive for osteocalcin & osteopontin are indicated by red arrowheads. NB, new bone; OS, osteoid; FM, fatty marrow. Image collected & cropped by CiteAb from the following publication (https://peerj.com/articles/3513), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Osteopontin/OPN Antibody (1B20) - BSA Free [NB110-89062] - Photomicrographs of immunostaining for osteocalcin & osteopontin in (A) Group I, (B) Group II (C) Group III at 6 weeks. The pictures are arranged by staining technique (columns) & the investigated treatment (rows). Areas that stained positive for osteocalcin & osteopontin are indicated by red arrowheads. NB, new bone; OS, osteoid; FM, fatty marrow. Image collected & cropped by CiteAb from the following publication (https://peerj.com/articles/3513), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Osteopontin/OPN (1B20) was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rat anti-Osteopontin/OPN (1B20) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB110-89062AF488) (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**

Anerillas, LO; Wiberg, M; Kingham, PJ; Kelk, P; Platelet lysate for expansion or osteogenic differentiation of bone marrow mesenchymal stem cells for 3D tissue constructs Regenerative therapy 2023-12-01 [PMID: 37588134] (Western Blot, Human)

G Feng, P Zhang, J Huang, Y Yu, F Yang, X Zhao, W Wang, D Li, S Sun, X Niu, L Chai, J Li Sequential Release of Panax Notoginseng Saponins and Osteopractic Total Flavone from Poly (L-Lactic Acid) Scaffold for Treating Glucocorticoid-Associated Osteonecrosis of Femoral Head Journal of functional biomaterials, 2023-01-04;14(1):. 2023-01-04 [PMID: 36662078] (Western Blot, Human)

Shen JF, Fan ZB, Wu CW et al. Sacubitril Valsartan Enhances Cardiac Function and Alleviates Myocardial Infarction in Rats through a SUV39H1/SPP1 Axis Oxidative Medicine and Cellular Longevity 2022-09-22 [PMID: 36193085] (Western Blot, Human)

V?ru? RM, Melinte PR, P□rvu AS et al. Calcium fructoborate coating of titanium-hydroxyapatite implants by chemisorption deposition improves implant osseointegration in the femur of New Zealand White rabbit experimental model Romanian Journal of Morphology and Embryology 2021-07-01 [PMID: 34171072] (Western Blot, Human)

Zhao, L;Wang, Z;Tan, Y;Ma, J;Huang, W;Zhang, X;Jin, C;Zhang, T;Liu, W;Yang, YG; IL-17A/CEBP?/OPN/LYVE-1 axis inhibits anti-tumor immunity by promoting tumor-associated tissue-resident macrophages Cell reports 2024-12-05 [PMID: 39643970]

Liao H, Zou Z, Liu W et Al. Osteopontin-integrin signaling positively regulates neuroplasticity through enhancing neural autophagy in the peri-infarct area after ischemic stroke Am J Transl Res 2022-11-15 [PMID: 36505285]

Abou-Rjeileh U, Dos Santos Neto JM, Chirivi M et al. Oleic acid abomasal infusion limits lipolysis and improves insulin sensitivity in adipose tissue from periparturient dairy cows Journal of dairy science 2023-04-25 [PMID: 37105874] (Simple Western, Bovine)

Details:

Dilutions 1:50

Li, Jiaxin, J Self-healing hybrid hydrogels with sustained bioactive components release for guided bone regeneration Research Square 2022-11-29 [PMID: 36814282] (ICC/IF, Mouse)

Park S, Park M, Saleh T, Lee B Autologous stromal vascular fraction-loaded hyaluronic acid/gelatin-biphasic calcium phosphate scaffold for bone tissue regeneration Mater Sci Eng C Mater Biol Appl 2022-02-12 [PMID: 35148865]

Kang H, Makkar P, Padalhin A et al. Comparative study on biodegradation and biocompatibility of multichannel calcium phosphate based bone substitutes Mater Sci Eng C Mater Biol Appl 2020-03-25 [PMID: 32204008]

Singh A, Kumar S, Acharya T et al. Application of nanohydroxyapatite-polysaccharide based biomaterial for bone cell mineralization in tissue engineering Materials Today Communications 2022-06-01 (ICC/IF, Human)

Fan C, Li Z, Ji Q et al. Carboxymethyl chitin or chitosan for osteoinduction effect on the human periodontal ligament stem cells Dental materials journal 2022-03-04 [PMID: 35249903] (ICC/IF, Human)

More publications at <a href="http://www.novusbio.com/NB110-89062">http://www.novusbio.com/NB110-89062</a>



#### **Procedures**

#### Western Blot Protocol Specific for Osteopontin Antibody (1B20)

Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 30 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.
- 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%.



#### Immunohistochemistry-Paraffin protocol for Osteopontin/OPN Antibody (NB110-89062)

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 4C.
- 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.

#### 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."





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## **Products Related to NB110-89062**

HAF007 Goat anti-Mouse IgG Secondary Antibody [HRP]

NB720-B Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]

NBP1-97005-0.5mg Mouse IgG1 Isotype Control (MG1)

NB110-89062AF488 Osteopontin/OPN Antibody (1B20) [Alexa Fluor® 488]

#### 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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