Product Datasheet

Osteopontin/OPN Antibody (1B20)
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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**Product Information**

- **Unit Size**: 0.1 ml
- **Concentration**: 1 mg/ml
- **Storage**: Store at 4°C short term. Aliquot and store at -20°C long term. Avoid freeze-thaw cycles.
- **Clonality**: Monoclonal
- **Clone**: 1B20
- **Preservative**: 0.05% Sodium Azide
- **Isotype**: IgG1
- **Purity**: Protein G purified
- **Buffer**: PBS pH 7.4

**Product Description**

- **Host**: Mouse
- **Gene ID**: 6696
- **Gene Symbol**: SPP1
- **Species**: Human, Rat, Rabbit
- **Reactivity Notes**: Human, Rat and Rabbit.
- **Immunogen**: Synthetic peptide corresponding to the C-terminus of human Osteopontin [Swiss-Prot# P10451].

**Product Application Details**

- **Applications**: Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, 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
- **Application Notes**: This Osteopontin (1B20) antibody is useful for Western blot, Immunohistochemistry on paraffin-embedded sections, Immunocytochemistry/Immunofluorescence and Immunoprecipitation. In Western blot multiple bands can be seen due to glycosylation and phosphorylation of the protein. Use in Immunohistochemistry-Frozen reported in scientific literature (PMID 24269728).

In Simple Western only 10 - 15 μL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.
Western Blot: Osteopontin/OPN Antibody (1B20) [NB110-89062] - Analysis of Osteopontin expression in U2OS whole cell lysate.

Immunocytochemistry/Immunofluorescence: Osteopontin/OPN Antibody (1B20) [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).


Simple Western: Osteopontin/OPN Antibody (1B20) [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.
Publications

AlyesSary AS, Yap AU, Othman Sa et al. Is there an optimal initial amount of activation for midpalatal suture expansion: A histomorphometric and immunohistochemical study in a rabbit model. J Orofac Orthop Apr 11 2018 12:00AM [PMID: 29644389] (Rabbit)


Details:
Osteopontin/OPN antibody was used for IHC-P application on sections from formalin-fixed paraffin embedded skin biopsies of males and female subjects with a diagnosis of calciphylaxis, which combines features of vascular thrombotic occlusion and endoluminal calcification.


Nguyen LT, Min YK, Lee BT. Nanoparticle Biphasic Calcium Phosphate loading on Gelatin-Pectin scaffold for improved bone regeneration Tissue Eng Part A. 2015 Jan 20 [PMID: 25602709] (IHC-P, Rabbit)

Details:
Osteopontin antibody used for Immunohistochemistry on the femur bone samples (collected after 6 and 12 weeks of implantation) from Rabbit.


Details:
Osteopontin antibody used for IHC-P in paraformaldehyde-fixed lung tissues from Wistar rats intratracheally instilled with vehicle control, L-SWCNT/single-wall carbon nanotubes or H-SWCNT at 90 days post-instillation. Antibody diluted in TBS and target detected using HRP-conjugated biotinylated secondary -DAB (Figure 7).


More publications at http://www.novusbio.com/NB110-89062
Procedures

Western Blot Protocol Specific for Osteopontin Antibody (1B20)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 30 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%.
Immunohistochemistry-Paraffin Embedded Sections Protocol (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 1:25,000 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."
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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