

Product Datasheet

Semaphorin 3B Antibody - BSA Free NB100-2218

Unit Size: 0.05 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB100-2218**Semaphorin 3B Antibody - BSA Free**

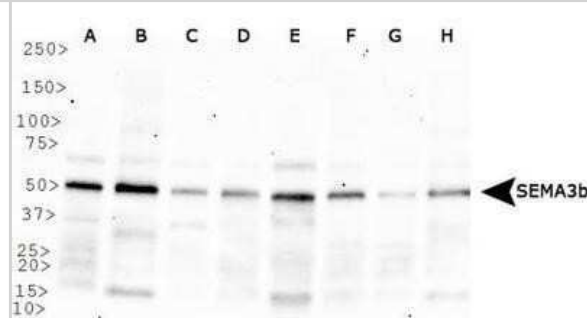
Product Information	
Unit Size	0.05 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit Semaphorin 3B Antibody - BSA Free (NB100-2218) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and Simple Western. Anti-Semaphorin 3B Antibody: Cited in 2 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	7869
Gene Symbol	SEMA3B
Species	Human, Mouse, Rat, Bovine, Canine, Primate
Immunogen	A synthetic peptide made to an internal portion of the human SEMA3B protein sequence (between residues 100-200). [UniProt# Q13214]

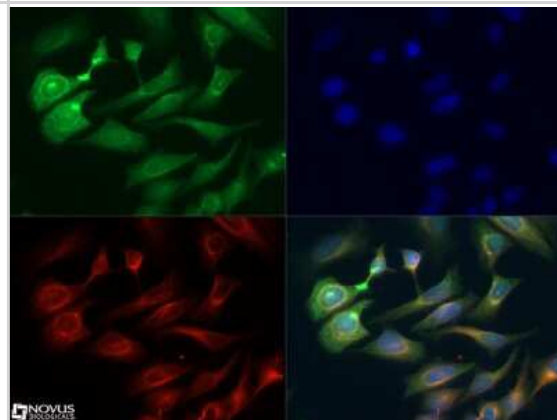
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:500-1:2000, Simple Western 1:25, Immunohistochemistry 1:100 - 1:200, Immunocytochemistry/ Immunofluorescence 1:40, Immunohistochemistry-Paraffin 1:100 - 1:200
Application Notes	<p>This SEMA3B antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and Immunohistochemistry paraffin embedded sections. In Western Blot, a band is seen at ~50 kDa, representing the secreted form of the protein and also a faint band at ~83 kDa, representing the pro-form of the protein. In ICC/IF, strong staining of endoplasmic reticulum and lighter signal in cytoplasm was observed in neuro2a cells. In IHC-P, staining was observed secreted, in the cytoplasm, and in the endoplasmic reticulum of mouse kidney tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>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 HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:25, apparent MW was 55 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

Images

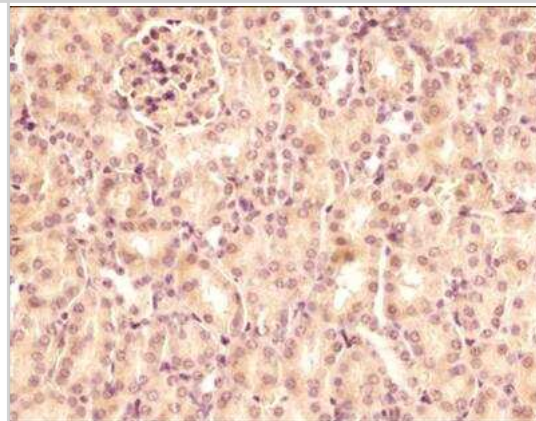
Western Blot: Semaphorin 3B Antibody [NB100-2218] - Western blot analysis of SEMA3B in A. HeLa WCE B. Ntera2 C. A431 D. HepG2 E. MCF7 F. NIH/3T3 G. PC12 H. Cos7



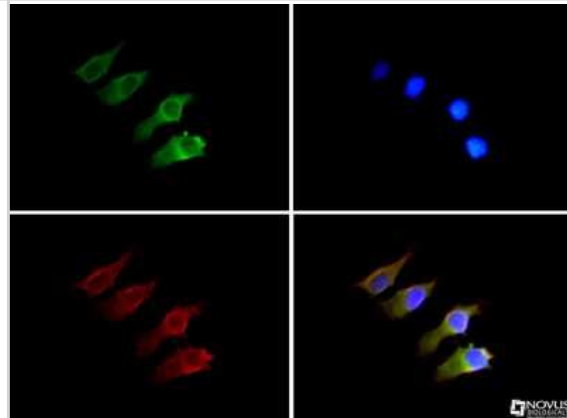
Immunocytochemistry/Immunofluorescence: Semaphorin 3B Antibody [NB100-2218] - SEMA3B antibody (1:50) was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). Image objective 40x.



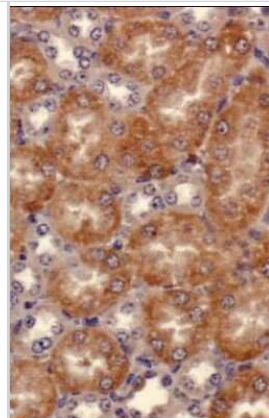
Immunohistochemistry-Paraffin: Semaphorin 3B Antibody [NB100-2218] - IHC analysis of a formalin fixed paraffin embedded tissue section of mouse kidney using 1:200 dilution of rabbit anti-Semaphorin 3B antibody. This antibody generated a weak to moderate cytoplasmic staining in the cells of various renal tubules and glomeruli.



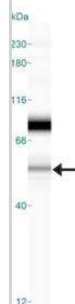
Immunocytochemistry/Immunofluorescence: Semaphorin 3B Antibody [NB100-2218] - SEMA3B antibody was tested in Neuro-2a cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



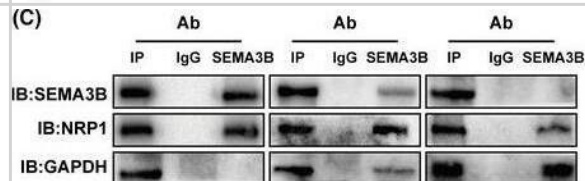
Immunohistochemistry: Semaphorin 3B Antibody [NB100-2218] - IHC analysis of SEMA3B in mouse kidney using DAB with hematoxylin counterstain.



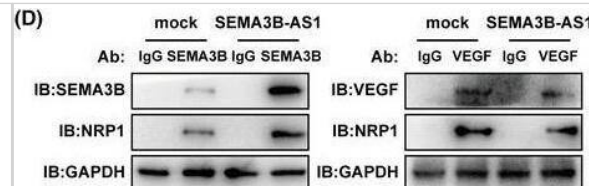
Simple Western: Semaphorin 3B Antibody [NB100-2218] - Simple Western lane view shows a specific band for SEMA3B in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



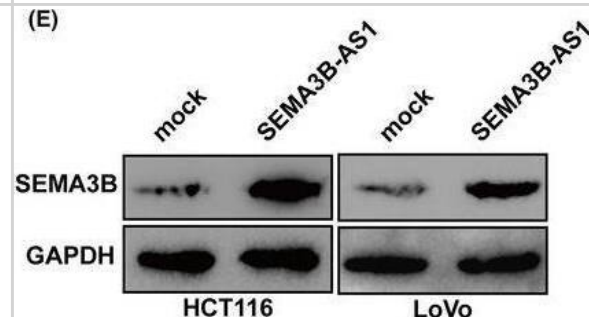
SEMA3B \square AS1 requires SEMA3B/NRP1 to suppress angiogenesis. (A) Protein network analysis of possible SEMA3B interacted proteins. The red square highlights NRP1. (B) SEMA3B and NRP1 colocalize in the blood vessels of colorectal carcinoma (CRC) patients. The scale bars indicate 100 and 20 μ m in pictures at 100 \times or 400 \times magnifications, respectively. (C) Both SEMA3B and vascular endothelial growth factor (VEGF) interact with NRP1, as analyzed by coIP. (D) NRP1 can bind with more SEMA3B and less VEGF after SEMA3B \square AS1 overexpression. (E–G) SEMA3B \square AS1 required SEMA3B/NRP1 to suppress the human umbilical vein endothelial cell (HUVEC) invasion (E), tubule formation in vitro (F), and angiogenesis in the chorioallantoic membrane (CAM) assay (G). Scale bars indicate 50 μ m (E) and 100 μ m (F), respectively. The yellow circles in (G) indicate locations where conditioned medium was used. (H) Schematic diagram showing the mechanism of action of SEMA3B \square AS1 in CRC. SEMA3B \square AS1 was downregulated in colorectal carcinoma and consequently decreased EP300 recruitment. In turn, the reduction in EP300 recruitment suppressed the accumulation of the active marker H3K9ac and repressed SEMA3B levels. Subsequently, the receptor NRP1 interacted with less SEMA3B, and then, the VEGF pathway was activated, which induced angiogenesis and colorectal carcinoma progression. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Source: Illustration created with BioRender (available online: <https://biorender.com/> (accessed on 15 June 2022)). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37701532>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



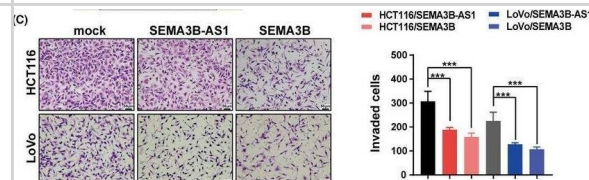
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SEMA3B, the sense \square cognate gene for SEMA3B \square AS1, is a key downstream target of SEMA3B \square AS1. (A) Genomic location of SEMA3B and SEMA3B \square AS1 from the ENCODE collection. Higher levels of epigenetic modification marks on histone 3 at lysine 9 (H3K9ac) and several transcription factor binding site uniform peaks of EP300 were observed within the SEMA3B promoter region. (B and C) The correlation between SEMA3B \square AS1 transcript levels and SEMA3B mRNA levels in colorectal carcinoma tissues was measured according to our data (n = 30; B) and The Cancer Genome Atlas (TCGA) cohort (n = 622; C). (D and E) SEMA3B \square AS1 regulated the expression of SEMA3B at the mRNA (D) and protein (E) levels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37701532>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



SEMA3B overexpression inhibits angiogenesis and the vascular endothelial growth factor (VEGF) pathway. (A) Gene set enrichment analysis (GSEA) showed the enrichment of the VEGF pathway in CRC cells with SEMA3B downregulation. (B) Overexpression of SEMA3B \square AS1 or SEMA3B increased the content of SEMA3B protein in the supernatant of colorectal cancer cells. (C) Colorectal carcinoma (CRC) cell supernatant overexpressing SEMA3B \square AS1 or SEMA3B inhibited the invasion ability of endothelial cells. Representative images (left) and the statistical analysis (right) are shown. Scale bars indicate 50 μ m. (D) CRC cell supernatant overexpressing SEMA3B \square AS1 or SEMA3B inhibited the tube formation of endothelial cells in vitro. Scale bars indicate 100 μ m. (E) The chorioallantoic membrane (CAM) assay was used to examine blood vessel formation after stimulation with the supernatants from the indicated cells. The yellow circles indicate locations where conditioned medium was used. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37701532>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Dziobek K, Oplawski M, Grabarek B et al. Expression of Semaphorin 3B (SEMA3B) in Various Grades of Endometrial Cancer Med. Sci. Monit. 2019-06-20 [PMID: 31217417] (IF/IHC, Human)

Fonseca FP, Bingle L, Santos-Silva AR et al. Semaphorins and neuropilins expression in salivary gland tumors J. Oral Pathol. Med. 2015-07-22 [PMID: 26199980] (IHC-P, Human)



Procedures

Western Blot protocol for SEMA3B Antibody (NB100-2218)

Semaphorin 3B Antibody:

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%.

*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 SEMA3B Antibody (NB100-2218)

Semaphorin 3B Antibody:

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 Semaphorin 3B Antibody (NB100-2218)**Semaphorin 3B Antibody:
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.

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Products Related to NB100-2218

NBL1-15798	Semaphorin 3B Overexpression Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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