Product Datasheet

Semaphorin 3B Antibody
NB100-2218

Unit Size: 0.05 ml

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

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Publications: 2

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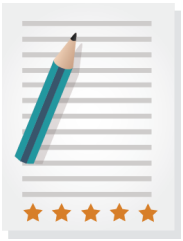
Updated 1/23/2024 v.20.1



NB100-2218

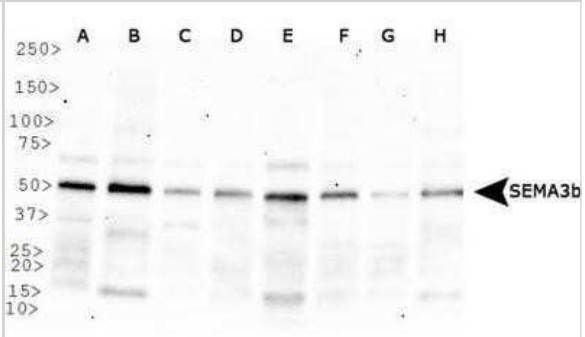
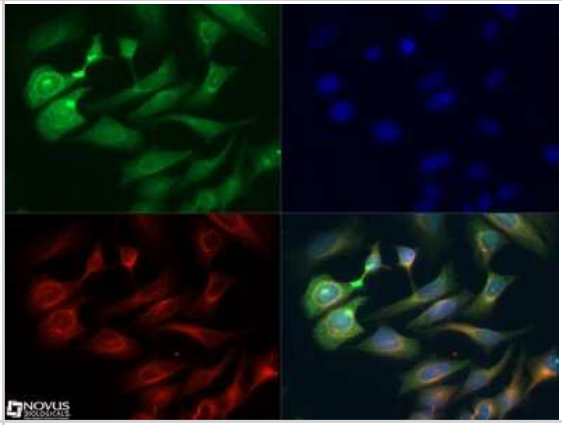
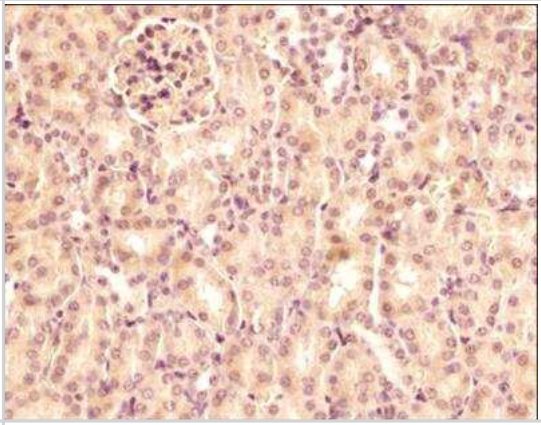
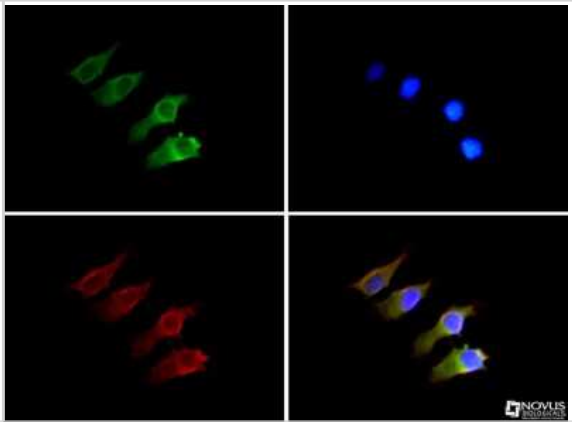
Semaphorin 3B Antibody

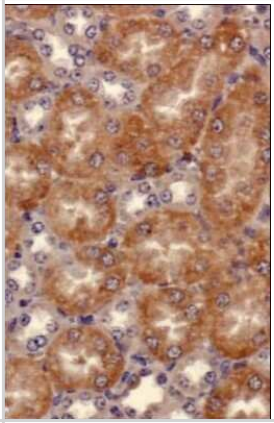
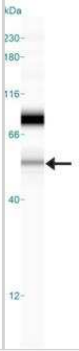
Product Information	
Unit Size	0.05 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine and 0.15M NaCl

Product Description	
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, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
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	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. 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: 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.	

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: https://www.novusbio.com/products/semaphorin-3b-antibody_nb100-2218
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: https://www.novusbio.com/products/semaphorin-3b-antibody_nb100-2218
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: https://www.novusbio.com/products/semaphorin-3b-antibody_nb100-2218
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.

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Products Related to NB100-2218

NBL1-15798	Semaphorin 3B Overexpression Lysate
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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