NEUROMICS

Semaphorin3B Data Sheet

Catalog Number:	RA25088	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human, Rat, Mouse, Primate, Cow, Dog
Immunogen Sequence:	A synthetic peptide made to an internal portion of the human SEMA 3B protein sequence (between residues 100-200).	**Format:**	Liquid. Tris-glycine, 150 mM NaCL and 0.05% Sodium Azide as a preservative. Concentration: 1.0 mg/ml.

Applications: Western Blot 1:5000
Immunohistochemistry: 1:100 (paraffin-embedded tissue).
Immunocytochemistry/Immunofluorescence 1:40

*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

Immunohistochemistry-Paraffin Embedded Sections
Note: SEMA3B protein is secreted and accumulates in the endoplasmic reticulum.
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 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.

Western Blot Protocol
*Note: 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***.**
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%.

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Image: SEMA3B antibody staining of Neuro-2a cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 594 (red).

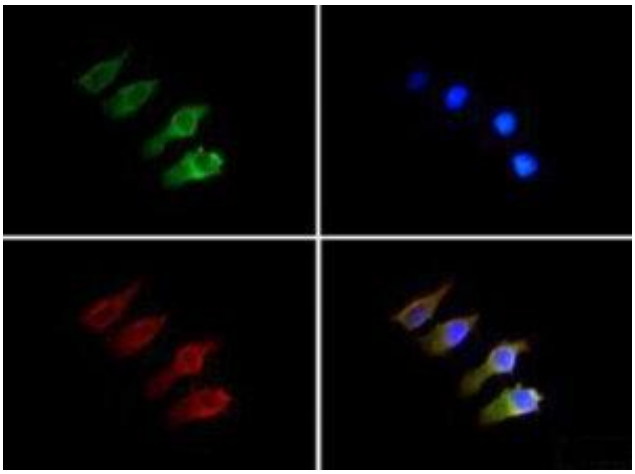
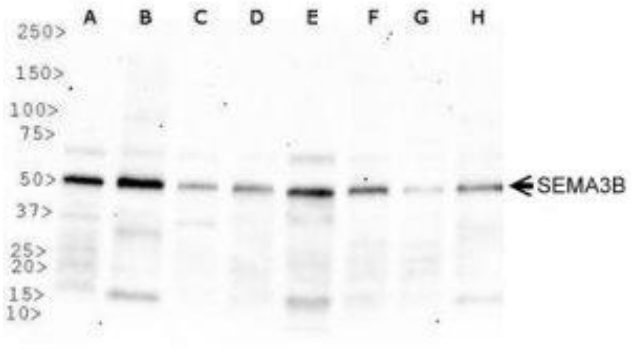


Image: Western blot analysis of SEMA3B in A. Hela WCE B. Ntera2 C. A431 D. HepG2 E. MCF7 F. NIH/3T3 G. PC12 H. Cos7.



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More Semaphorins and Plexins

Name	Catalog #	Type	Species	Applications	Size	Price
Plexin A3	GT15211	Goat IgG	H; M; R	IHC; WB; E	100 ug	$365
Semaphorin 3C	GT15077	Sheep IgG	M	IHC; WB; E	100 ug	$345
Semaphorin 5A	GT41026	Goat IgG	H	IHC	100 ug 100 ug Blocking Peptide	$250 $145
Semaphorin 6A	GT15056	Chicken IgY			100 ug	$365
Semaphorin 6A	MO15023	Rat IgG	M	IHC; WB; E	500 ug	$325
Semaphorin 6B	GT15165	Goat IgG	H; M	IHC; WB; E	100 ug	$365
Semaphorin 7A	GT15076	Goat IgG	M	WB; E	50 ug	$365
Semaphorin 7A	MO15041	Rat IgG	H; M	WB; E	500 ug 100 ug	$325 $89

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