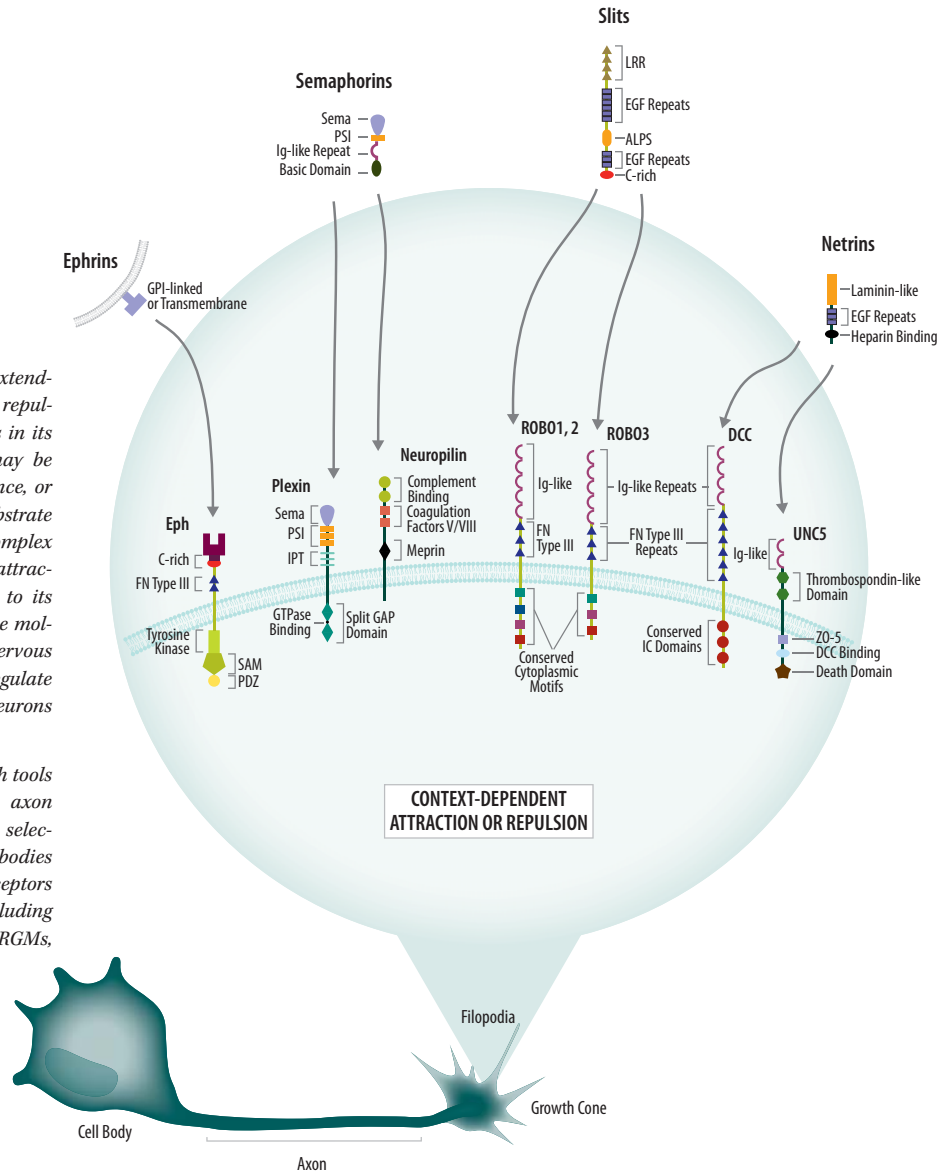


Axon Guidance

The growth cone at the tip of an extending axon is exquisitely sensitive to repulsive and attractive guidance cues in its environment. These molecules may be diffusible and work from a distance, or be bound to a membrane or substrate and work at close range. It is the complex integration of these repulsive and attractive signals that guide the axon to its appropriate target. Axon guidance molecules play critical roles during nervous system development and may regulate the regenerative capacity of neurons during nervous system disease.

R&D Systems offers many research tools to study molecules involved in axon guidance. These include a wide selection of active proteins and antibodies designed for the ligands and receptors of several protein families including Netrins, Semaphorins, Ephrins, RGMs, Slits, BMPs, Wnts, and more.

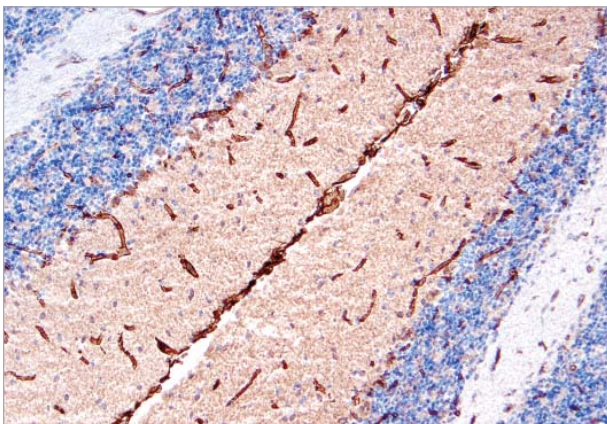
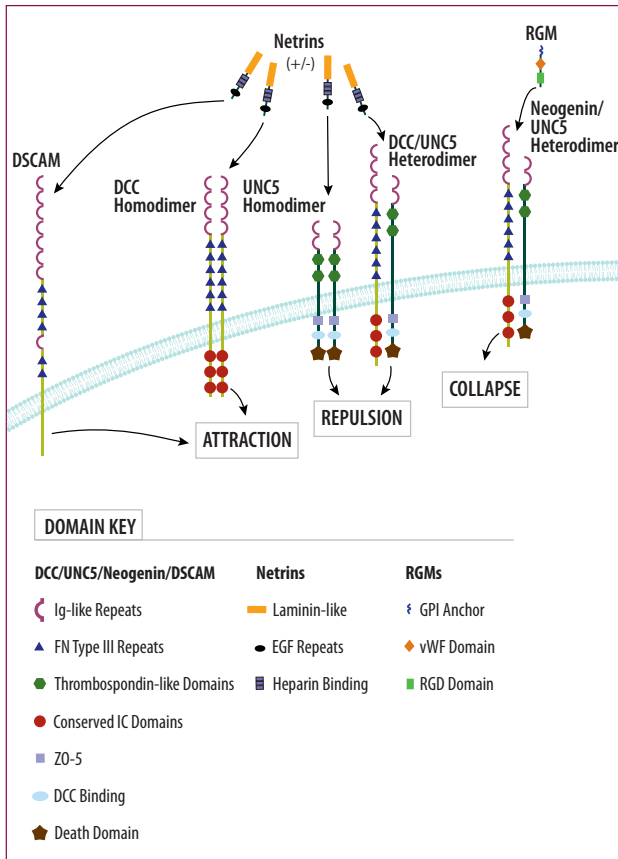


Netrins, RGMs, & their Receptors

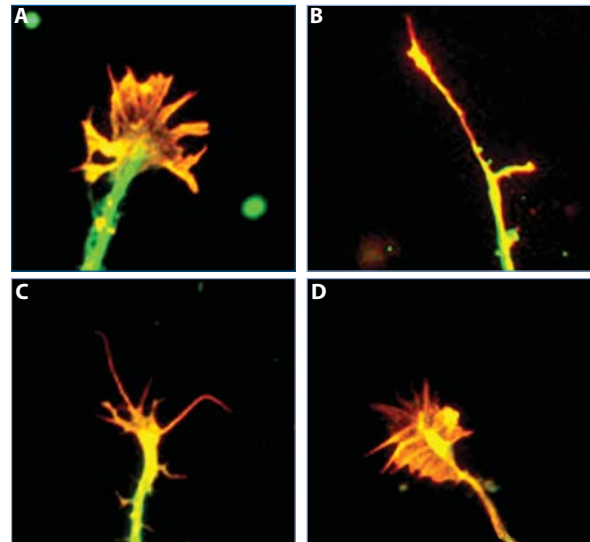
Netrins are a small family of laminin-related molecules that includes both secreted (Netrin-1, -3, and -4) and membrane-associated proteins (Netrin-G1, -G2). Netrins have been shown to bind to a complex combination of receptors that affect the elicited response. Netrins bind to UNC5 and DCC family receptors to mediate context-dependent repulsive (UNC5) or attractive (DCC) effects on axon guidance. UNC5 and DCC receptors form homodimers and heterodimers to regulate signaling. DSCAM has also been reported as a Netrin receptor, potentially acting alone or in combination

with DCC. Neogenin is another putative Netrin receptor that shares structural similarity to DCC and interacts with UNC5. This receptor combination has also been shown to bind to members of the repulsive guidance molecule family (RGM-A, RGM-B, RGM-C) and specifically, mediate the growth cone collapsing activity of RGM-A.

For more information on Netrin-related products, please visit our website at www.RnDSystems.com/go/Netrins



Netrin-4 Expression in Mouse Cerebellum. Netrin-4 was detected in a cryostat tissue section of mouse cerebellum using anti-mouse Netrin-4 polyclonal antibody (Catalog # AF1132). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).



RGM-A-induced Growth Cone Collapse Requires UNC5H2/UNC5b. **A:** An embryonic rat (E19-20) cortical growth cone in culture treated with control siRNA. **B:** Treatment with recombinant mouse RGM-A (Catalog # 2458-RG; 2 µg/mL) induces growth cone collapse. **C:** A neuronal growth cone treated with UNC5H2/UNC5b siRNA alone has no effect on morphology. **D:** UNC5H2/UNC5b knockdown with siRNA prevents RGM-A-induced growth cone collapse. Figure adapted with permission from Hata, K. et al. (2008) *J. Cell Biol.* **184**:737.

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
DCC	M	H M	
DSCAM		H	
Neogenin	M	M	
Netrin-1	M Ch	M Ch	
Netrin-2	Ch	Ch	
Netrin-4	H M	H M	
Netrin-G1a	M	M	
Netrin-G2a		M	
NGL-1	H	H	
Nope	M	M	M
RGM-A	H M	H M Ch	
RGM-B	H M	H M	
RGM-C	H M	H M	
UNC5H1	R	R	
UNC5H2	R	R	
UNC5H3	H	H	
UNC5H4	H	H	

KEY: H: Human, M: Mouse, R: Rat, Ch: Chicken

Ephrins & Eph Receptors

Ephrins and their tyrosine kinase receptors, Ephs, are divided into two classes, the Ephrin-A and Ephrin-B ligand families. Ephrin-A ligands are anchored to the membrane via GPI linkage and preferentially bind EphA receptors, while Ephrin-B ligands are transmembrane proteins that preferentially interact with EphB receptors. Most Eph receptors are not ligand specific. Ephrins and their Eph receptors have the unusual capacity of bidirectional signaling, involving the activation of signal transduction

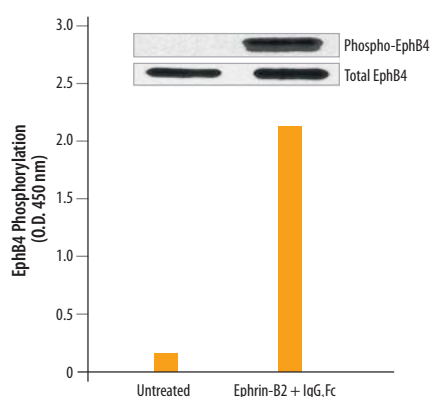
pathways in both ligand- and receptor-expressing cells. Ephrins/Ephs may have a number of context-dependent activities including mediating attraction, repulsion, cell adhesion, or migration. Among their many roles, Ephrins/Ephs regulate topographic mapping along the anterior-posterior axis of the superior colliculus and guidance at the spinal cord midline.

For more information on Ephrin-Eph-related products, please visit our website at www.RnDSystems.com/go/Ephrins

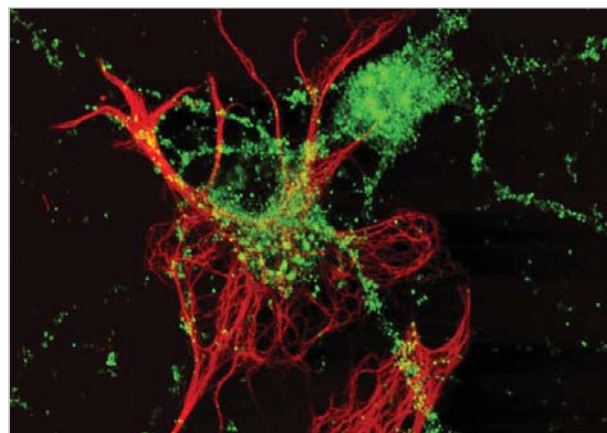
Eph Receptors	Ephrin Ligands
EphA1	Ephrin-A1 (low); Ephrin-B1
EphA2	Ephrin-A1, -A3, -A4, -A5
EphA3	Ephrin-A2, -A3, -A4, -A5
EphA4	Ephrin-A1, -A4, -A5; -B2; Ephrin-A2, -A3 (low)
EphA5	Ephrin-A1, -A2, -A3, -A4, -A5
EphA6	Ephrin-A1
EphA7	Ephrin-A1, -A2, -A3, -A4, -A5
EphA8	Ephrin-A1, -A2, -A3, -A4, -A5
EphA10	Unknown

EphB1	Ephrin-B1, -B2, -B3
EphB2	Ephrin-A5
EphB3	Ephrin-B1, -B2
EphB4	Ephrin-B2
EphB5	Unknown
EphB6	Unknown

Ligand Binding Specificities for Eph Family Receptors. The ligand binding specificities for different Eph receptors are shown. The information in the table was adapted from Surawska, H. et al. (2004) *Cytokine Growth Factor Rev.* 15:419.



EphrinB2-induced EphB4 Phosphorylation Assessed using the DuoSet® IC ELISA Development System. T47D human breast ductal carcinoma cells were left untreated or treated with recombinant mouse Ephrin-B2/Fc Chimera (Catalog # 496-EB) and human IgG, Fc (Catalog # 110-HG) to induce clustering. EphB4 tyrosine phosphorylation was assessed using the human Phospho-EphB4 DuoSet IC ELISA Development System (Catalog # DYC4057; bar graph). For comparison the same lysates were also assessed for phosphorylated and total EphB4 using Western blot.



Ephrin-A2 Expression in Rat Embryonic Neurons. Ephrin-A2 was detected in rat embryonic hippocampal neurons using anti-mouse Ephrin-A2 polyclonal antibody (Catalog # AF603) followed by FITC-conjugated anti-goat secondary antibody (green). Astrocytes were stained using an anti-GFAP antibody (red).

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
Eph	H M R		
EphA1	H M	H M	H
EphA2	H M	H M	H
EphA3	M	M	
EphA4	M	M	
EphA5	H M R	M R	H M
EphA6	H M	M	
EphA7	M	M	
EphA8	M	M	
EphA10	H		
EphB1	R	R	
EphB2	H M	M	
EphB3	H M	M	
EphB4	H M	H M	H
EphB6	H M	H M	
Ephrin	H M		
Ephrin-A1	M	M	
Ephrin-A2	M	M	
Ephrin-A3	H	H	
Ephrin-A4	H M	H M	
Ephrin-A5	H	H	
Ephrin-B		H M R Ch X	
Ephrin-B1	M	M	
Ephrin-B2	M Z	M Z	
Ephrin-B3	H	H	

KEY: H: Human, M: Mouse, R: Rat, Ch: Chicken, X: Xenopus, Z: Zebrafish

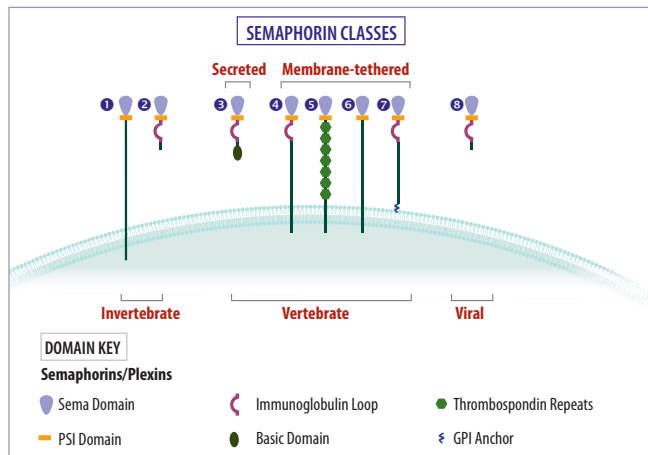
Semaphorins, Neuropilins, & Plexins

The Semaphorins constitute a large family of secreted and membrane-tethered molecules that can be divided into eight classes according to their structure and species of origin. Classes 1 and 2 are found in invertebrates, classes 3 through 7 are found in vertebrates, and class 8 is viral. Of the vertebrate Semaphorins, class 3 Semaphorins are secreted, classes 4, 5, and 6 are transmembrane proteins, and class 7 molecules are GPI-anchored. Two distinct transmembrane receptor families, Neuropilins and Plexins, have been identified as Semaphorin receptors. Neuropilins (NRP-1 and NRP-2) provide binding specificity for class 3 Semaphorins, while Plexins serve as

functional receptors for membrane-associated Semaphorins, and as signaling mediators for class 3 Semaphorins. Semaphorin-Plexin signaling regulates cytoskeletal reorganization and cell adhesion, and is involved in processes such as axon guidance, angiogenesis, hematopoiesis, organogenesis, and immune cell regulation.

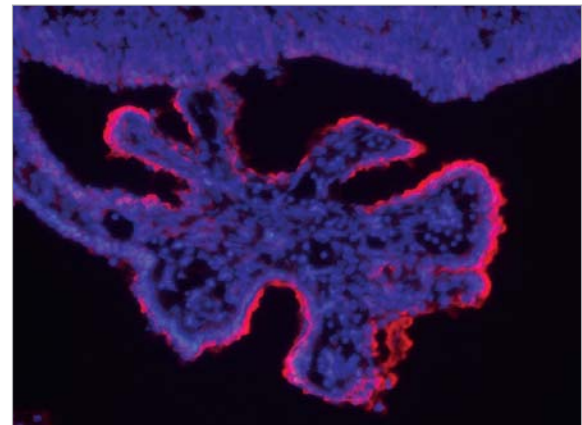
Neuropilins and Plexins are highly expressed on neurons. Classically described as collapsing factors and mediators of axon repulsion *in vitro*, Semaphorins regulate axon branching and prevent axons from entering certain regions of the nervous system during development *in vivo*.

For more information on Semaphorin-, Neuropilin-, or Plexin-related products, please visit our website at www.RnDSystems.com/go/Semaphorins

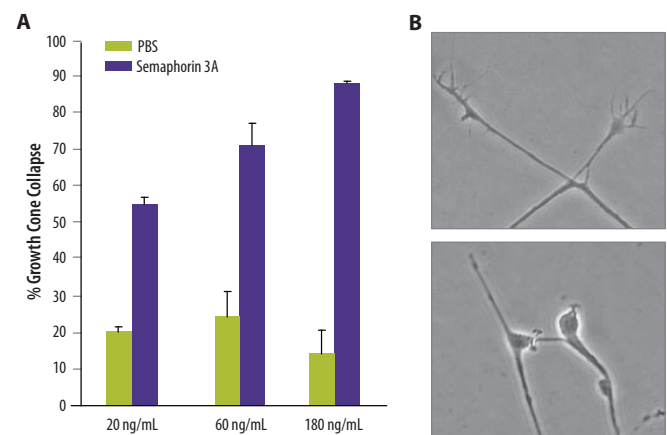


MOLECULE	PROTEINS	ANTIBODIES
AGF R/c-MET	H M Ca	H M Ca
Neuropilin-1/BDCA4	H R	H M R
Neuropilin-2	H R	H R
Plexin A1		M
Plexin A3		M
Plexin B1		H
Plexin B2		H
Plexin B3	H	
Plexin C1	H	H M
Plexin D1	H	H
Semaphorin 3A	H M	H
Semaphorin 3B	M	
Semaphorin 3C	H	M
Semaphorin 3E	H M	H
Semaphorin 3F	M	H M
Semaphorin 4A	H	
Semaphorin 4B		H
Semaphorin 4D/CD100		M
Semaphorin 6A	H	H M
Semaphorin 6B	H	H M
Semaphorin 6C		H M
Semaphorin 6D		H
Semaphorin 7A		H M
TEM7		H
TIM-2		M

KEY: H: Human, M: Mouse, R: Rat, Ca: Canine



Plexin B2 Expression in Embryonic Mouse Choroid Plexus. Plexin B2 was detected in embryonic mouse choroid plexus using anti-human Plexin B2 polyclonal antibody (Catalog # AF5329). Cells were stained with NorthernLights™-557 donkey anti-sheep IgG (Catalog # NL010; red) secondary antibody and counterstained with DAPI (blue).



Semaphorin 3A-induced Growth Cone Collapse. **A.** E8 chick dorsal root ganglion explants, grown in the presence of recombinant human β -NGF (Catalog # 256-GF), were incubated with PBS or with increasing concentrations of recombinant human Semaphorin 3A (Catalog # 1250-S3). The percent of growth cone collapse was assessed following a thirty minute incubation (Collapsed = Less than 3 filopodia; Non-collapsed = 3 filopodia or more). **B.** A fully extended chick dorsal root ganglion growth cone grown in the presence of recombinant human β -NGF (Catalog # 256-GF) was left untreated (top) or treated with recombinant human Semaphorin 3A (Catalog # 1250-S3; bottom). Treatment with recombinant Semaphorin 3A induced growth cone collapse.

Morphogens as Axon Guidance Cues

A morphogen is classically defined as a signaling molecule that elicits different cellular responses depending on its concentration. More specifically, morphogens are secreted molecules that drive the organization of regional groups of cells into patterns. Until recently, morphogens and guidance molecules were considered to be structurally and functionally distinct. Now, however, evidence suggests that select, early-expressed

morphogens can be temporally “recycled” and serve as axon guidance cues. Members of the hedgehog, bone morphogenetic protein (BMP), and Wnt families have all been implicated as axon guidance factors.

For more information on Morphogen-related products, please visit our website at www.RnDSystems.com/go/Morphogen

WNTS AND RELATED MOLECULES

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
APC		H	
Axin-1		H M R	
β-Catenin		H M R X	H
Dishevelled-1		H	
Dishevelled-2		H	
Dishevelled-3		H	
Dkk-1	H M R	H M R	H M
Frizzled-1	M	H M	
Frizzled-2	M	M	
Frizzled-3		H M	
Frizzled-4	M	H M	
Frizzled-5	H	H	
Frizzled-6		H M	
Frizzled-7	M	H M	
Frizzled-8	M	M	
LRP-6	H M	H M	
Norrin	H M	H M	
Pygopus-1		M	

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
Pygopus-2		H	
ROR1 Receptor Tyrosine Kinase		H	
RTK-like Orphan Receptor 2/ROR2		H	
R-Spondin 3	H	H M	
Ryk		H M	
sFRP-1*	H	H	
sFRP-2	M	M	
sFRP-3	H M	H M	
sFRP-4	H	H	
TCF-7/TCF-1		H	
Wnt-2		H	
Wnt-2b		M	
Wnt-3a	H M	M	
Wnt-5a	M	M	
Wnt-7a	H	H	
Wnt-10b		M	

*U.S. Patent # 6,479,255 and patents pending.

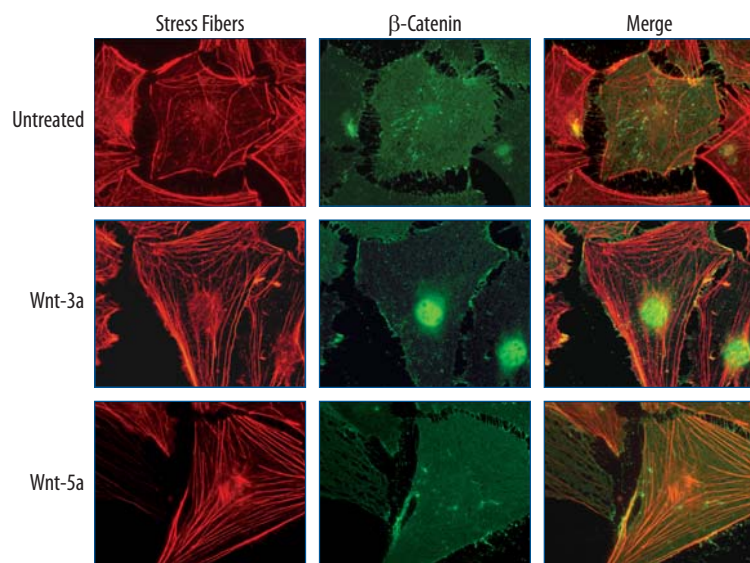
BMPS AND RELATED MOLECULES

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
BMP-2	H Z	H Z	H M R
BMP-2/BMP-7 Heterodimer	H	H	
BMP-2/BMP-4		H	
BMP-2a	Z		
BMP-3	H	H	
BMP-3b/GDF-10	H	H	
BMP-4	H M Z	H Z	H
BMP-4/BMP-7 Heterodimer	H		
BMP-5	H	H	H
BMP-6	H	H	H
BMP-7	H M	H	H
BMP-8		H	
BMP-9	H M	H	
BMP-10	H	H	
BMP-15/GDF-9B	H	H	
BMPR-1A/ALK-3	H M	H	
BMPR-1B/ALK-6	H M	H M	
BMPR-II	H	H	

HEDGEHOG AND RELATED MOLECULES

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
Patched		M	
Patched 2		H	
Desert Hedgehog	H M	M	
Indian Hedgehog	M	M	
Sonic Hedgehog	H M	H M	M
HIP	M	M	M
GLI-1		H M	
GLI-2		H M	
GLI-3		H M	
BOC	H	H M	
CDO	H	H M	
DISP1		H	

KEY: H: Human, M: Mouse, R: Rat, X: *Xenopus*, Z: Zebrafish



Wnt-induced Stress Fiber Formation and Nuclear β-Catenin Accumulation. R&D Systems recombinant mouse Wnt-3a (Catalog # 1324-WN) and Wnt-5a (Catalog # 645-WN) promote stress fiber formation in NIH-3T3 mouse embryonic fibroblast cells, while only Wnt-3a promotes nuclear β-Catenin accumulation. Please visit our website for information about our new high purity human Wnt-3a (Catalog # 5036-WNP). Images Courtesy of Dr. Raymond Habas, Robert Wood Johnson School of Medicine, Piscataway, NJ.

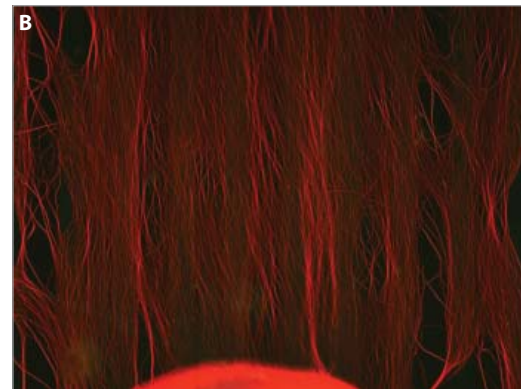
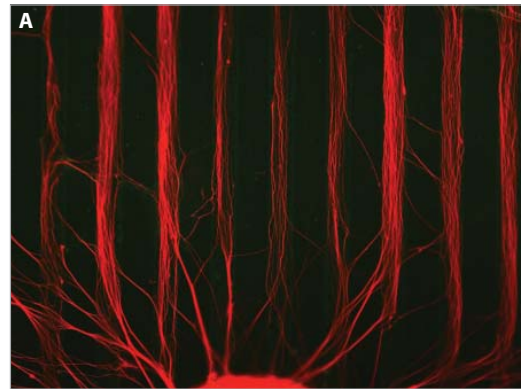
Cell Adhesion: Extracellular Matrix, Cadherins, CAMs, Reelin, & More

Growing axons experience spatiotemporal changes in adhesion that affect their ability to reach a specific target. These interactions can be between adjacent cells (cell-cell adhesion), or between cells and the extracellular matrix. Cell adhesion can affect axon guidance by enhancing or inhibiting outgrowth, fasciculation, and/or regeneration. Cell adhesion molecules (CAMs) of the Ig superfamily, extracellular matrix-associated proteins, integrins, cadherins, and proteoglycans are among the adhesion-related factors reported to affect axonal outgrowth and guidance.

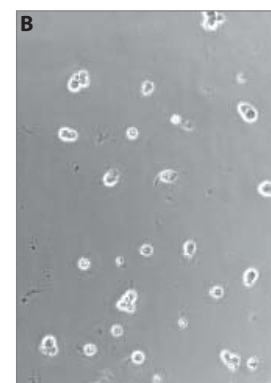
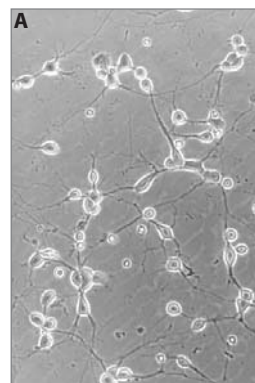
For a complete listing of R&D Systems cell adhesion-related products, please see our website at www.RnDSystems.com/go/CellAdhesion

MOLECULE	PROTEINS	ANTIBODIES
AMIGO		H
AMIGO2		H M
AMIGO3		M
ApoE R2	H	
CD44	H	H Ca
CHL1/L1CAM-2	H M	H M
Contactin-1	H	H
Contactin-2/TAG1	H M	H
Contactin-3	H	H M R
Contactin-4	H M	H
Contactin-5	H	H
DSCAM		H M
DSCAM-L1		H
Fibronectin	H B	H
F-spondin	H	H
Kilon/Neurotractin	H	H M
Integrins	Please see our website for a large selection of Integrin-related research tools	
Laminin		H M R Ch
Mindin		H
N-Cadherin	H	H
NCAM-1/CD56	H	H
NCAM-L1	H	H
Neurocan	M	M R
Neurofascin	R	
Neuroglycan		H M R
Neurotrimin		H
NrCAM	H	H
Reelin	M	M
R-Spondin-1	H M	H M
R-Spondin-2	H	H
R-Spondin-3	H	H M
Tenascin C	H	H M
Tenascin R	H	H M R
VLDL R	M	M

KEY: H: Human, M: Mouse, R: Rat, B: Bovine, Ca: Canine, Ch: Chicken



Retinal Ganglion Cell (RGC) Axon Fasciculation Requires Homophilic Contactin-2/TAG1 Interactions. Retinal explants were isolated from E14.5 TAG1^{+/+} or TAG1^{-/-} embryos. *In vitro* assays of RGC axonal growth were performed on glass coverslips coated with alternating stripes of Laminin and Contactin-2/TAG1 (Catalog # 1714-CN). **A:** TAG1^{+/+} axons prefer Contactin-2/TAG1 to Laminin. **B:** Contactin-2/TAG1^{-/-} axons display no preference between Contactin-2/TAG1- and Laminin-coated stripes. RGC axons were immunolabeled with anti-neurofilament antibodies (red). *Data Courtesy of Dr. Jean-Léon Thomas, Université Pierre et Marie Curie, Paris.*



Neurofascin-induced Neurite Outgrowth. **A:** Recombinant rat Neurofascin (Catalog # 3235-NF) immobilized on a microplate promotes neurite outgrowth in rat cortical neurons. **B:** Cortical neurons cultured under the same conditions in the absence of Neurofascin exhibit little outgrowth.

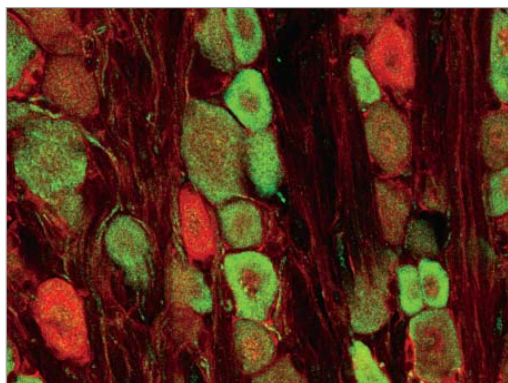
Neurotrophic Factors, Myelin-associated Factors, and their Receptors

Neurotrophic factors are involved in an array of nervous system activities including regulating neuronal survival, neurite outgrowth, and synaptic plasticity. These include members of the GDNF family: GDNF, Artemin, Persephin, and Neurturin. This family signals through a multimolecular complex that includes receptors of the GFR- α family and RET. Other neurotrophic factors include the neurotrophins: NGF, BDNF, NT-3, and NT-4. Pro-neurotrophins preferentially bind the receptor NGF R/p75^{NTR} in combination with the co-receptor Sortilin to promote apoptosis, while Trk tyrosine kinase receptors (TrkA, TrkB, TrkC) preferentially bind mature neurotrophins and promote survival. There is also an intriguing relationship between NGF R/p75^{NTR} and myelin-associated factors shown to inhibit neurite outgrowth and regeneration. NGF R/p75^{NTR}, Nogo R, Lingo-1, and/or the TNF receptor superfamily member, TROY, may act in a receptor complex mediating activities of the outgrowth-inhibiting proteins Nogo-A, MAG, and OMgp.

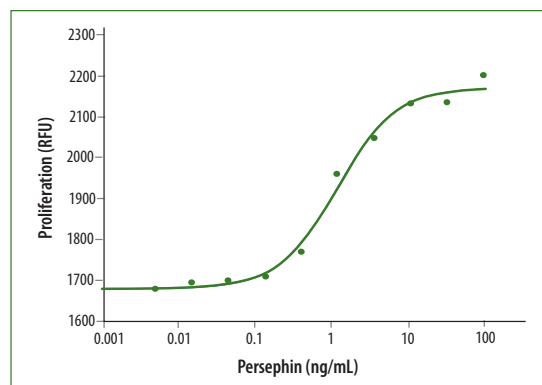
Please see our website at www.RnDSystems.com/go/NeurotrophicFactors for a detailed listing of related products

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
Artemin	H M	H M	
BDNF	H	H	H
GDNF	H R	H R	
GFR α -1	H R	H R	
GFR α -2	H M	H M	
GFR α -3	H M	H M	
GFR α -4		H M	
NGF R/p75 ^{NTR}	H M	H M	
Lingo-1		H	
Lingo-2		H	
MAG	R	R	R
MBP		H M R B	
MOG		H M	
β -NGF	H M R	H R	H R
NgR2		H	
NgR3		H	
Nogo A	H R	H R	
Nogo C	H		
Nogo Receptor	H M	H M	
NT-3	H	H	H
NT-4	H	H	H
O1		H M R Ch	
O4		H M R Ch	
OMgp	H M	H M	
Neurturin	H M	H M	
Persephin	H M	H M	
Ret	H M	H M	
ROCK1	H		
ROCK2		H M R	
Sortilin	H M	H M	
TrkA	H R	H R	H
TrkB	H M	H M	H
TrkC	H M	H M	H
TROY	H M	H M	M

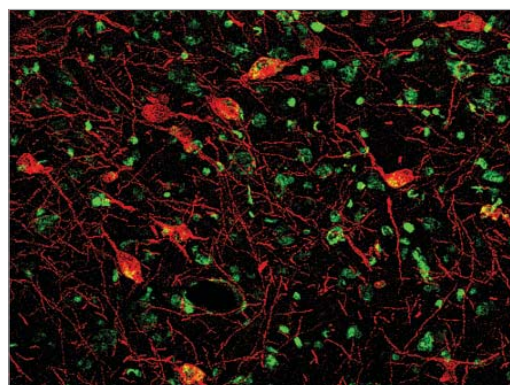
KEY: H: Human, M: Mouse, R: Rat, B: Bovine, Ch: Chicken



GFR α -1 and GDNF Expression in Rat Dorsal Root Ganglion. GFR α -1 and GDNF were detected in rat dorsal root ganglion using biotinylated anti-rat GFR α -1 polyclonal antibody (Catalog # BAF560; red) and goat anti-human GDNF polyclonal antibody (Catalog # AF-212-NA; green). Tissues were stained with streptavidin-conjugated CyTM3 and a FITC-conjugated anti-goat secondary antibody.



Persephin-induced Proliferation. Human thyroid carcinoma (TT) cells proliferate in a response to increasing concentrations of recombinant mouse Persephin (Catalog # 2479-PS). Proliferation was assessed using Resazurin (Catalog # AR002) fluorescence.



NGF Receptor (NGF R)/p75^{NTR} in Mouse Brain. NGF R/p75^{NTR} was detected in a cryostat tissue section of mouse brain using anti-mouse NGF R/p75^{NTR} affinity-purified polyclonal antibody (Catalog # AF1157; red). Tissues were counterstained with FluoroNisslTM green (green).

Cy is a trademark of GE Healthcare
FluoroNissl is a trademark of Molecular Probes



USA & Canada
R&D Systems, Inc.
 614 McKinley Place NE, Minneapolis, MN 55413
 Tel: (800) 343-7475 (612) 379-2956
 Fax: (612) 656-4400
 info@RnDSystems.com

PRSRT STD
 U.S. POSTAGE
PAID
 R&D SYSTEMS

Change Service Requested



Printed on recyclable paper 10% post consumer waste.

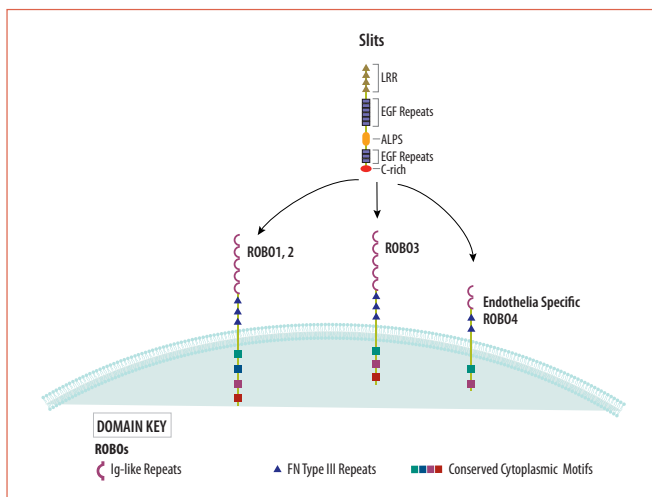
R&D Systems is a registered trademark of TECHNE Corporation.

FL102_axonguidance_DEC

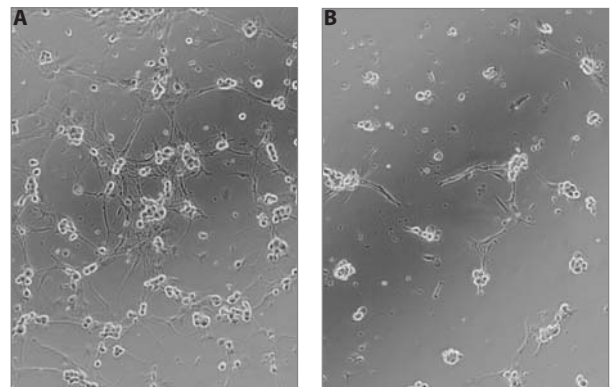
Slit Proteins & ROBO Receptors

The Slit family of proteins consists of three members (Slit1-3) that signal by binding to one of four Roundabout (ROBO1-4) receptors. Slits are large, secreted glycoproteins that are subject to proteolytic cleavage resulting in fragments with variable activities. ROBO1, ROBO2, and ROBO3 are predominantly expressed in the nervous system and Slit-ROBO interactions have been shown to regulate axon repulsion and neuronal outgrowth.

For more information on products related to the Slit or ROBO families, please visit our website at www.RnDSystems.com/go/SlitROBO



Slit Proteins Direct Cell Migration by Binding to ROBO Family Receptors. The Slit family of proteins binds to members of the ROBO family of receptors to initiate signaling pathways that affect cell motility. This binding is mediated by the leucine-rich repeat (LRR) region of the Slit proteins and the immunoglobulin-like (Ig-like) repeats of the ROBO family of receptors.



Slit2 Enhances Neurite Outgrowth. Cultured chick dorsal root ganglion neurons were grown in the presence of recombinant human β -NGF (Catalog # 256-GF; 50 ng/mL), with (A) or without (B) recombinant mouse Slit2 (Catalog # 5444-SL; 12 μ g/mL). The presence of the Slit2 protein significantly enhanced neurite outgrowth.

MOLECULE	PROTEINS	ANTIBODIES	ELISAS
ROBO1	R	R	R
ROBO2	H	H	
ROBO3	H M	H	
ROBO4		H M	
Slit1	M		
Slit2	M		
Slit3	M	M	

KEY: H: Human, M: Mouse, R: Rat