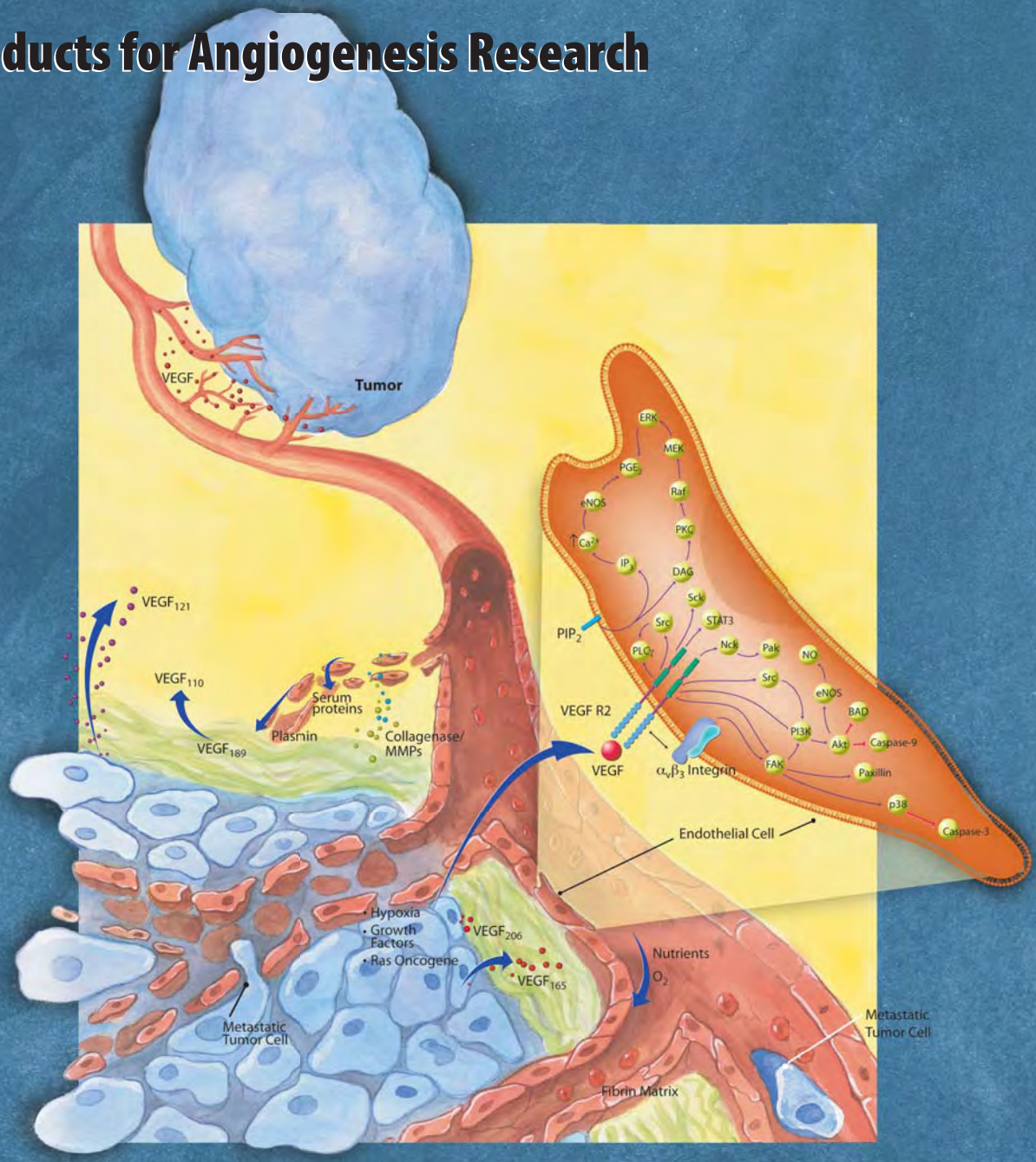
R&D Systems Tools for Cell Biology Research™

Products for Angiogenesis Research



R&D SYSTEMS®

Introduction

Blood vessels form a patterned network of arteries, capillaries, and veins that transport different substances and cells bidirectionally throughout the vertebrate body. These vessels form *de novo* during embryonic development from angioblast precursor cells or in adult organisms from endothelial progenitor cells by the process of vasculogenesis. The primary vasculature is modified by angiogenesis, which involves the sprouting of new capillaries from pre-existing vessels. Angiogenesis requires degradation of the vascular basement membrane, followed by activation of endothelial cell proliferation, migration, and tube formation. Newly formed vessels are subsequently stabilized by the recruitment of pericytes and vascular smooth muscle cells. Angiogenesis is largely regulated by the balance of pro- and anti-angiogenic factors. These factors, associated with endothelial cells, the extracellular matrix, and tumor cells, regulate endothelial cell activities and vessel stabilization.

Angiogenesis is a fundamental phenomenon in physiological processes such as embryonic development, wound healing, and pregnancy. It is typically downregulated in healthy adults, but can be induced by pathological conditions associated with inflammation or hypoxia such as atherosclerosis, cancer, rheumatoid arthritis, Crohn's disease, or diabetes. Under these conditions, the balance between pro- and anti-angiogenic factors is disrupted and the "angiogenic switch" is turned on. Multiple studies now suggest that suppression of angiogenesis can inhibit the progression of many of these conditions.

R&D Systems offers a wide selection of products for angiogenesis-related research including proteins, antibodies, ELISAs, and kits for cell selection and multi-analyte profiling.

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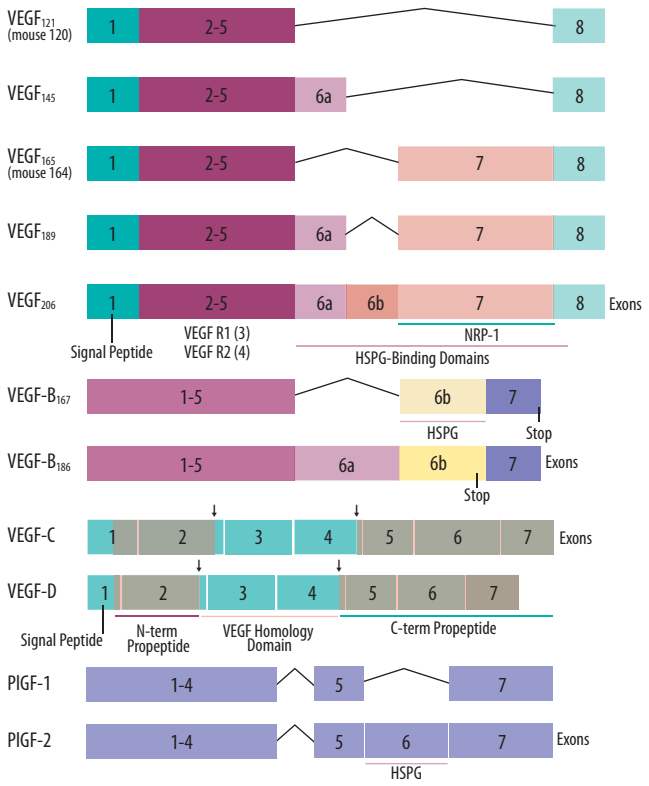
I. VEGF/PDGF Family

VEGF Family

Vascular endothelial growth factor (VEGF) and its receptors are primary regulators of vasculogenesis and angiogenesis. Although a number of other cellular factors are also involved in these processes, many are directly controlled by VEGF, or act to enhance or inhibit VEGF-induced signaling pathways. VEGF signaling is required for physiological angiogenesis associated with development, wound healing, and pregnancy, as well as pathological angiogenesis associated with the progression of a variety of disease conditions. Understanding the pathways that VEGF activates and the mechanisms by which it can be regulated are areas of intense research.

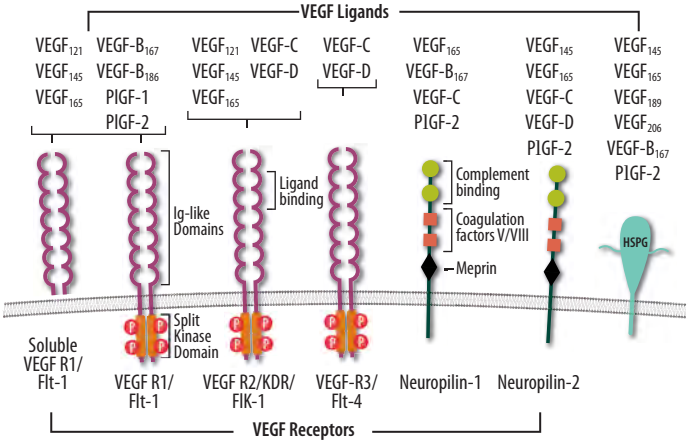
The VEGF gene family consists of six members, VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, viral VEGF-E, and placental growth factor (PlGF). Human VEGF has eight exons and can form multiple alternatively spliced isoforms with different biological properties including $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$. Of these, $VEGF_{165}$ is the most common and has been found to be upregulated in a number of human tumors. Other less prevalent forms also exist: $VEGF_{148}$, $VEGF_{162}$, and $VEGF_{183}$. Additionally, two alternatively spliced variants of VEGF-B, $VEGF\text{-}B_{167}$ and $VEGF\text{-}B_{186}$, and four isoforms of PlGF (PlGF-1-4) have been reported.



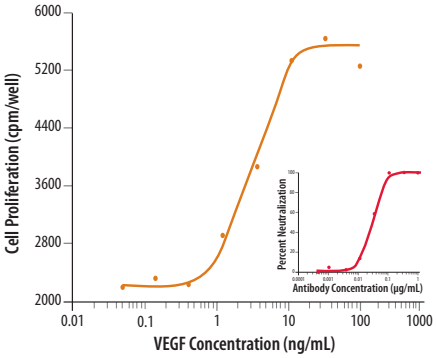
Alternative Splicing Generates Multiple Isoforms of Human VEGF and PlGF. Alternative splicing of members of the VEGF gene family including VEGF, VEGF-B, and PlGF, generates multiple cellular isoforms with different receptor- and HSPG-binding properties. For VEGF, this generates proteins with molecular masses of 121, 145, 165, 189, and 206 kDa denoted as $VEGF_{121}$, $VEGF_{145}$, etc, as well as other less common forms. While exons 1-5 of VEGF are conserved in all isoforms, exons 6 and 7, which encode two HSPG binding domains, are variable and determine whether a particular VEGF isoform is bound to the cell surface or is diffusible. In addition, two isoforms of VEGF-B and four isoforms of PlGF are produced by alternative splicing. Like VEGF, PlGF can also bind to VEGF receptors to regulate angiogenesis, but the role of VEGF-B in angiogenesis is not currently well understood. Only one isoform of VEGF-C and VEGF-D have been identified to date. These proteins are the primary mediators of lymphangiogenesis. (Note: the $VEGF_{148}$, $VEGF_{162}$, $VEGF_{165b}$, and $VEGF_{183}$ splice variants, as well as viral VEGF-E, and PlGF-3 and -4 family members are not shown.)

The biological effects of different isoforms of VEGF are mediated by binding to one of three transmembrane receptor tyrosine kinases expressed on endothelial cells: VEGF R1/Flt-1, VEGF R2/KDR/Flk-1, and VEGF R3/Flt-4. Ligand binding promotes receptor dimerization, autophosphorylation, and activation of intracellular signaling pathways. Some forms of VEGF can also bind to heparan sulfate proteoglycans (HSPGs), and to Neuropilin-1 or Neuropilin-2, two receptors known for their involvement in axon guidance as Semaphorin co-receptors. The Neuropilin co-receptors and HSPG regulate VEGF ligand/receptor interactions and can enhance downstream signaling.

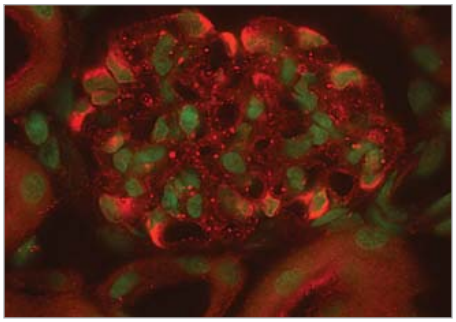
Activation of different VEGF receptors has distinct cellular effects. VEGF binding to VEGF R2 is primarily responsible for stimulating endothelial cell proliferation, migration, and tube formation associated with angiogenesis, while VEGF-C and VEGF-D promote lymphangiogenesis by binding to VEGF R3. The third VEGF receptor, VEGF R1, has been characterized as a decoy receptor that can negatively regulate VEGF signaling by preventing VEGF from binding to VEGF R2. This receptor may also mediate pro-angiogenic effects under pathological conditions such as rheumatoid arthritis and cancer. Inhibition of VEGF signaling may help to prevent the pathogenesis of multiple diseases in which angiogenesis is required for disease progression. At the same time, mechanisms to enhance VEGF signaling are being investigated to promote angiogenesis in ischemic diseases.



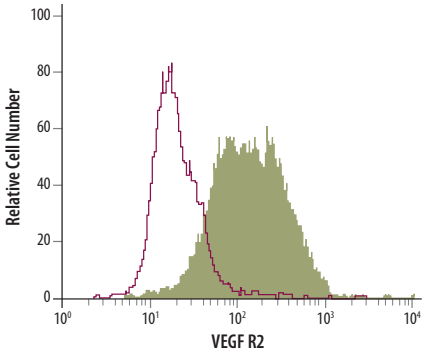
Receptor Binding Specificities for Isoforms of VEGF and PlGF. Human VEGF isoforms bind to three receptor tyrosine kinases expressed on endothelial cells including VEGF R1, VEGF R2, and VEGF R3. VEGF R1 and VEGF R2 mediate physiological and pathological angiogenesis, while VEGF R3 regulates lymphangiogenesis. Specific VEGF isoforms can also bind to Neuropilin-1 and Neuropilin-2 co-receptors, and heparan sulfate proteoglycans (HSPG) that act in conjunction with VEGF receptors to modulate VEGF signaling. In addition, isoforms of PlGF bind to VEGF R1, Neuropilin-1, and Neuropilin-2.



VEGF-Induced HUVEC Proliferation & Antibody Neutralization. Human umbilical vein endothelial cells (HUVECs) were incubated with increasing concentrations of recombinant human $VEGF_{165}$ (Catalog # 293-VE) and cell proliferation was assessed by measuring ^{3}H-thymidine incorporation (orange line). The effect induced by 10 ng/mL VEGF was neutralized in a dose-dependent manner using anti-human $VEGF_{165}$ polyclonal antibody (Catalog # AF-293-NA; inset).



VEGF Expression in Mouse Kidney. VEGF was detected in frozen sections of mouse kidney glomeruli using anti-mouse VEGF polyclonal antibody (Catalog # AF-493-NA; red). Tissues were counterstained (green).



Detection of VEGF R2 by Flow Cytometry. Human umbilical vein endothelial cells (HUVECs) were stained with PE-conjugated anti-human VEGF R2/KDR monoclonal antibody (Catalog # FAB357P; filled histogram) or with PE-conjugated mouse IgG_1 isotype control (Catalog # IC002P; open histogram).

PRODUCTS FOR VEGF-RELATED RESEARCH

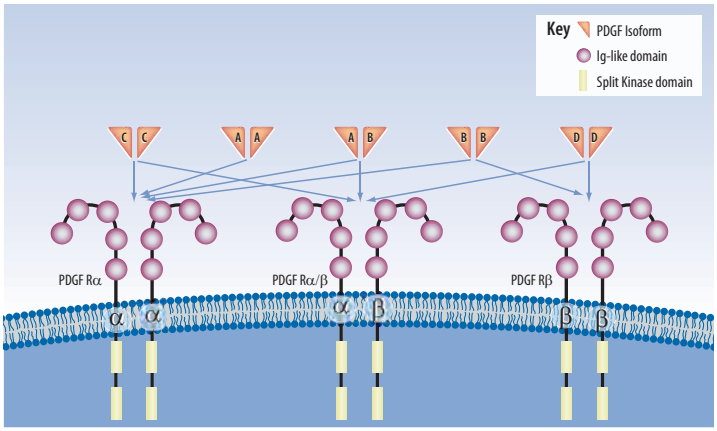
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS	FLOW CYTOMETRY KITS
Neuropilin-1/BDCA4	H R	H M R				
Neuropilin-2	H R	H R				
PlGF	H	H	H	H		
PlGF-2	M	M	M			
VEGF	H M R Ca Z	H M R Ca Z	H M R Ca	H M R		H
VEGF/PlGF Heterodimer	H	Hw	H			
VEGF-B	H M	H M				
VEGF-C	H	H	H			
VEGF-D	H M	H M	H M	H		
VEGF R		H				
VEGF R1/Flt-1	H M	H M	H M			
VEGF R2/KDR/Flk-1	H M	H M	H M		H	
VEGF R3/Flt-4	H M	H M	H M			

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

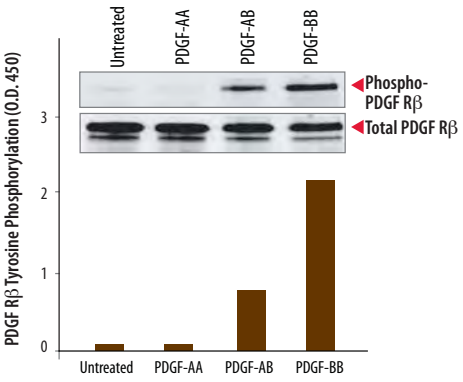
PDGF Family

Platelet-derived growth factor (PDGF) is a mitogenic factor that is produced by a variety of cells including endothelial and vascular smooth muscle cells. PDGFs are produced from four different genes and secreted as disulfide-linked homodimers with identical subunits, PDGF-A (PDGF-AA), PDGF-B (PDGF-BB), PDGF-C (PDGF-CC), or PDGF-D (PDGF-DD), or as PDGF-AB heterodimers. PDGFs differentially bind homodimer and heterodimer combinations of two receptor tyrosine kinases, PDGF Rα and PDGF Rβ, to activate intracellular signaling cascades that are required for multiple developmental processes including angiogenesis. In the development of vessel walls, PDGF-B/PDGF Rβ has been shown to be required for the recruitment and spreading of pericytes and vascular smooth muscle cells. Additionally, recent studies suggest that PDGF-A/PDGF Rα signaling may be involved in creating a VEGF-rich microenvironment for angiogenic tumor growth. Cancer researchers are attempting to simultaneously target both the VEGF and PDGF signaling pathways.

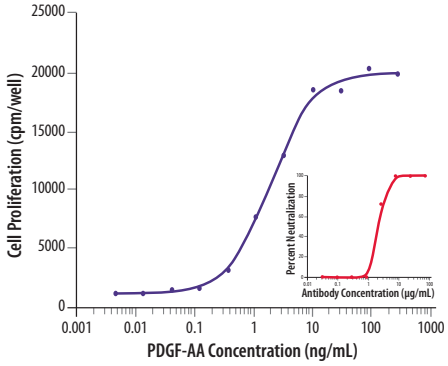
For more information on PDGF-related products, please visit our website at www.RnDSystems.com/go/PDGF



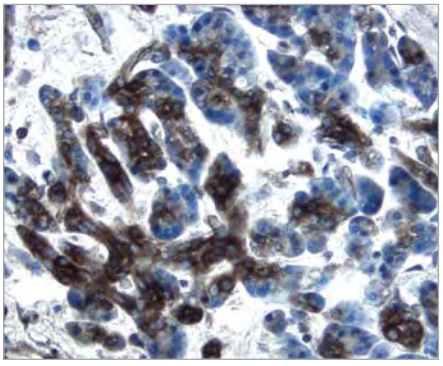
Ligand-Receptor Interactions for Members of the PDGF Family. PDGF isoforms (PDGF-A, -B, -C, -D) form disulfide-linked homodimers (PDGF-AA, -BB, -CC, -DD) and heterodimers (PDGF-AB) that differentially bind to homodimer and heterodimer combinations of two receptor tyrosine kinases, PDGF Rα and PDGF Rβ. Ligand binding promotes PDGF R autophosphorylation, which initiates downstream signaling pathways involved in a wide range of developmental and pathological functions including angiogenesis. [Figure adapted from Li, X. & U. Eriksson (2003) Cytokine Growth Factor Rev. **14**:91.]



Ligand-induced PDGF Rβ Phosphorylation Assessed using the DuoSet® IC ELISA Development System. Cell lysates from immortalized human fibroblasts, untreated or treated with recombinant human PDGF-AA (Catalog # 221-AA), PDGF-AB (Catalog # 222-AB), or PDGF-BB (Catalog # 220-BB), were assessed for PDGF Rβ phosphorylation using the human Phospho-PDGF Rβ DuoSet IC ELISA Development System (Catalog # DYC1767; bar graph). The results are consistent with immunoprecipitation/Wesern blot analysis performed using the same lysates (inset).



PDGF-Rα-mediated Mouse Fibroblast Proliferation & Antibody Neutralization. NR6R-3T3 mouse fibroblasts were treated with increasing concentrations of recombinant human PDGF-AA (Catalog # 221-AA) and cell proliferation was assessed by monitoring ^{3}H-thymidine incorporation (purple line). The stimulatory effect induced by 10 ng/mL PDGF-AA was neutralized by pre-incubating the cells with increasing concentrations of anti-mouse PDGF Rα polyclonal antibody (Catalog # AF1062) prior to the addition of the recombinant protein (inset).



PDGF-C Expression in Human Pancreatic Cancer. PDGF-C was detected in paraffin-embedded human pancreatic cancer tissue sections using anti-human PDGF-C polyclonal antibody (Catalog # AF1560). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue)

PRODUCTS FOR PDGF-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
PDGF	H P	H Ms		
PDGF-A		H		
PDGF-AA	H R	H R Ms	H M	H
PDGF-AB	H R	H Ms	H M R	
PDGF-B		H Ms		
PDGF-BB	H R	H	H M R	H
PDGF-C		H M		

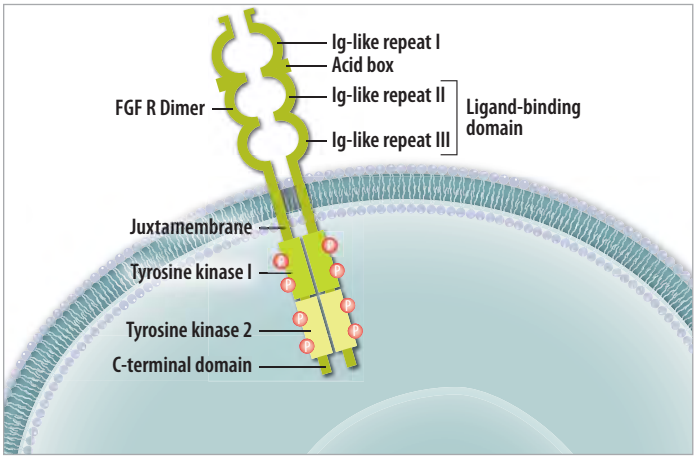
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
PDGF-CC	H M			
PDGF-D		H		
PDGF-DD	H			
PDGF Rα	H M	H M	H	
PDGF Rβ	H M	H M	H M	

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

II. FGF Family

Fibroblast Growth Factors (FGFs) constitute a large family of proteins involved in many aspects of development including cell proliferation, growth, and differentiation. FGF acidic (FGF-1) and FGF basic (FGF-2) are two of the most intensely studied members of the FGF family of ligands, which consists of 22 different proteins in mice and humans. Both FGF acidic and FGF basic promote growth, differentiation, and migration of endothelial cells. The activities of the FGF proteins are mediated by one of four receptor tyrosine kinases (FGF R1-4), which exhibit alternative splicing in the Ig-like repeat domains and may require a co-receptor for activation in a cell- and context-dependent manner. FGF R5 is a decoy receptor that binds to FGF ligands, but does not propagate a signal.

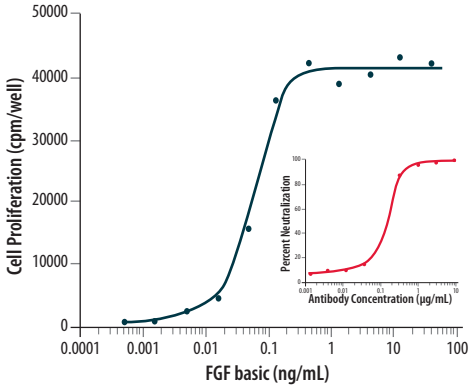
FGFs bind to heparan sulfate proteoglycans (HSPGs) and FGF receptor tyrosine kinases to activate intracellular signaling pathways initiated by receptor dimerization, autophosphorylation, and the recruitment and docking of SH2 domain-containing proteins. The FGF family has been implicated in several aspects of angiogenesis including endothelial cell migration, proliferation, morphogenesis, maturation, vessel stabilization, and regulation of extracellular matrix degradation. Recent studies suggest that FGF functions upstream of VEGF to promote angiogenesis. FGF basic stimulates the expression of VEGF in endothelial cells, and inhibition of FGF signaling negatively regulates angiogenesis with correlative downregulation of VEGF. Likewise, inhibition of VEGF signaling prevents FGF-induced angiogenesis. In addition, FGF basic also mediates PDGF-induced vessel stabilization by upregulating the expression of PDGF R on endothelial cells to render them more sensitive to PDGF-B-induced mural cell recruitment.



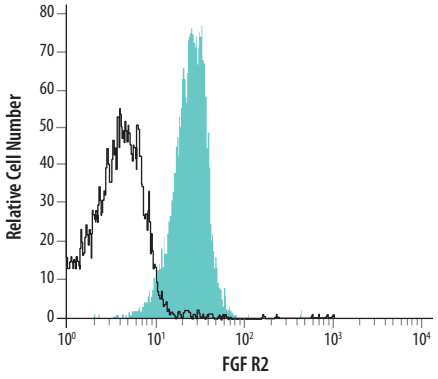
Structural Domains of FGF Receptors. Fibroblast growth factor (FGF) receptors are characterized by three extracellular Ig-like repeat domains, with Ig-like domains II and III forming the ligand-binding segment. Upon ligand binding, FGF receptors dimerize in conjuction with heparan sulfate moieties. Receptor dimerization leads to autophosphorylation on multiple intracellular tyrosine residues. FGF R phosphorylation promotes the binding of adaptor proteins, which activate downstream signaling cascades.

			FGF RECEPTORS						
SUBFAMILY	LIGAND	ALTERNATE NAME	R1b	R1c	R2b	R2c	R3b	R3c	R4
FGF-1	FGF-1	FGF acidic	▪	▪	▪	▪	▪	▪	▪
	FGF-2	FGF basic	▪	▪		▪		▪	▪
FGF-4	FGF-4	K-FGF, hst-1		▪		▪		▪	▪
	FGF-5	HBGF-5		▪		▪			
	FGF-6	HBGF-6		▪		▪			▪
FGF-7	FGF-3	int-2	▪		▪				
	FGF-7	KGF			▪				
	FGF-10	KGF-2	▪		▪				
	FGF-22		▪		▪				
FGF-8	FGF-8a	AIGF							
	FGF-8b	AIGF		▪		▪		▪	▪
	FGF-8e	AIGF						▪	▪
	FGF-8f	AIGF				▪		▪	▪
	FGF-17			▪		▪		▪	▪
	FGF-18					▪		▪	▪
FGF-9	FGF-9	GAF				▪	▪	▪	▪
	FGF-16					▪	▪	▪	▪
	FGF-20			▪	▪	▪	▪	▪	▪
FGF-11	FGF-11	FHF-3							
	FGF-12	FHF-1							
	FGF-13	FHF-2							
	FGF-14	FHF-4							
FGF-19	FGF-19*	FGF-15 (mouse)		▪		▪		▪	▪
	FGF-21*			▪		▪		▪	▪
	FGF-23*			▪		▪		▪	▪

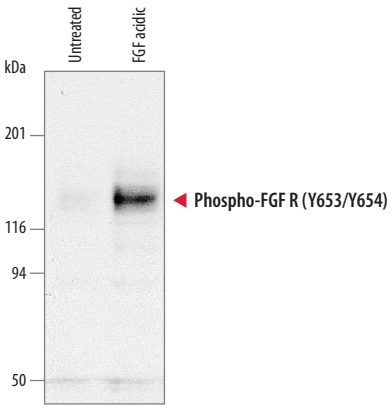
Ligand Binding Specificities for FGF Family Receptors. The ligand binding specificities for different FGF receptors are shown. * Indicates that the ligand requires a Klotho cofactor for signaling.



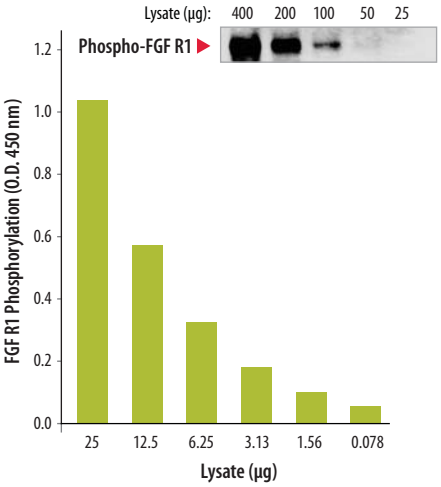
FGF basic-Induced Mouse Fibroblast Proliferation & Antibody Neutralization. NR6R-3T3 mouse fibroblasts were treated with increasing concentrations of bovine FGF basic (Catalog # 133-FB) and cell proliferation was assessed by measuring ^{3}H-thymidine incorporation (green line). The stimulatory effect induced by 0.5 ng/mL FGF basic was neutralized by pre-incubating the protein with increasing concentrations of anti-human FGF basic polyclonal antibody (Catalog # AF-233-NA) prior to its addition to quiescent confluent cultures of NR6R-3T3 fibroblasts (inset).



Detection of FGF R2 by Flow Cytometry. U937 human leukemic monocyte lymphoma cells were stained with APC-conjugated anti-human FGF R2 (Catalog # FAB684A; filled histogram). Staining with an APC-conjugated isotype control antibody (Catalog # IC002A; open histogram) highlights the specificity of the FGF R2 antibody.



Detection of Phosphorylated FGF R on Y653/Y654 by Western Blot. Cell lysates from Kato III human stomach cancer cells, untreated or treated with 100 ng/mL recombinant human FGF acidic (Catalog # 232-FA) for 10 minutes were immunoblotted using anti-human Phospho-FGF R (Y653/Y654) polyclonal antibody (Catalog # AF3285).



FGF R1 Phosphorylation in Transfected CHO-S Cells Assessed using the DuoSet® IC ELISA Development System. Cell lysates prepared from Chinese hamster ovary cells (CHO-S) transfected with human FGF R1 were serially diluted and assessed for FGF R1 phopshorylation using the human Phospho-FGF R1 DuoSet IC ELISA Development System (Catalog # DYC5079; bar graph) or by IP/Western blot (top). IP/Western blots were performed using anti-human FGF R1 monoclonal antibody and anti-mouse agarose for immunoprecipitation followed by detection using HRP-conjugated anti-Phospho-Tyrosine monoclonal antibody (Catalog # HAM1676).

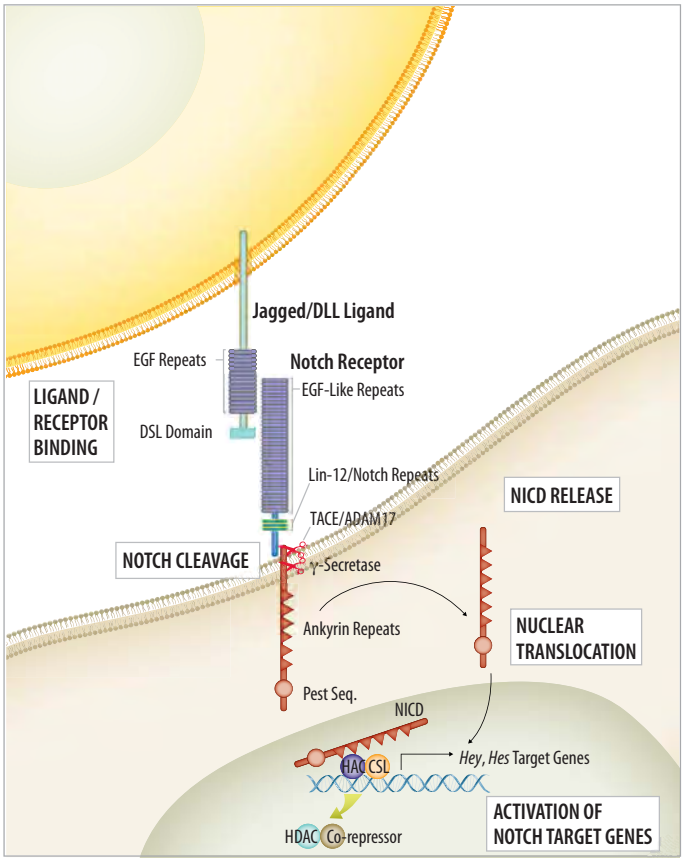
PRODUCTS FOR FGF-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
α2-Macroglobulin	H	H		
FGF acidic	H M B	H B	H	H
FGF acidic	H			
FGF basic	H M R B	H B	H	H
FGF-3	H	H		
FGF-4	H	H	H	
FGF-5	H	H		
FGF-6	H M	H	H	
KGF/FGF-7	H M Ca	H Ca	H	
FGF-8	H M	H M		
FGF-9	H	H	H	
FGF-10	H	H		
FGF-11		H		
FGF-12	H	H		
FGF-13		H		
FGF-16	H	H		
FGF-17	H	H		
FGF-19	H	H	H	
FGF-20	H	H		
FGF-21	H	H M		
FGF-22	H	H		
FGF-23	H M	H M		
FGF-BP	H R	H R		
FGF R1-4		H		
FGF R1	H	H	H	
FGF R1α	H			
FGF R1β	H			
FGF R2	H M	H M	H	
FGF R2α	H	H	H	
FGF R2β	H M			
FGF R3	H M	H M	H	
FGF R4	H	H M	H	
FGF R5/FGFRL1		H M		
FRS2		H M R	H M R	
Klotho	H M	M		
Klotho β	M	M		
Pentraxin 3/TSG-14	H M	H M	H M	
SPRY3		H		

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

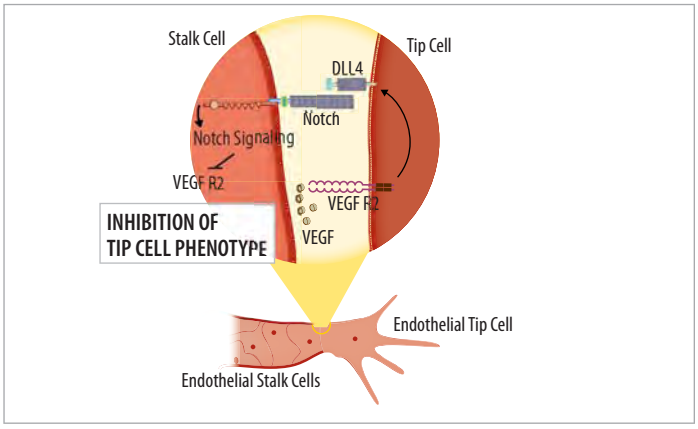
III. Delta/Notch Family

Notch family receptors interact with membrane-associated ligands on adjacent cells to regulate a number of early developmental processes, including vascular morphogenesis. Mammals have four Notch receptors (Notch-1-4) consisting of extracellular and intracellular fragments that associate noncovalently to form heterodimers at the cell surface. Notch receptors bind to ligands of the Jagged (Jagged 1, Jagged 2) and Delta-like gene families (DLL1, DLL3, DLL4). Notch activation results in sequential proteolysis of Notch by TNF-converting enzyme (TACE/ADAM17) and Presenilin-dependent γ-secretase. These cleavage events promote the release of the Notch intracellular domain (NICD), which translocates to the nucleus to regulate the transcription of Notch target genes.

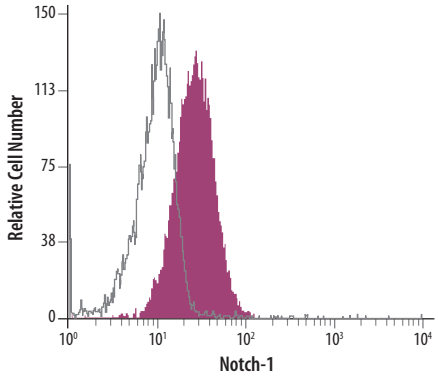


Notch Receptors Are Activated by Jagged or DLL Ligands on Adjacent Cells. Members of the Jagged (Jagged 1, Jagged 2) or Delta-like (DLL1, DLL3, DLL4) ligand families bind to membrane-associated, heterodimeric Notch receptors (Notch1-4) on adjacent cells. Following ligand binding, Notch is proteolytically cleaved by TACE/ADAM17 and Presenilin-dependent γ-Secretase. These proteolytic cleavage events release the Notch intracellular domain (NICD), which translocates to the nucleus. In the nucleus, NICD binds to the CSL protein, converting it from a transcriptional co-repressor to a co-activator complex. In addition, NICD recruits a histone acetyltransferase (HAC), and displaces a histone deacetylase (HDAC)/Co-repressor complex to activate Notch target genes.

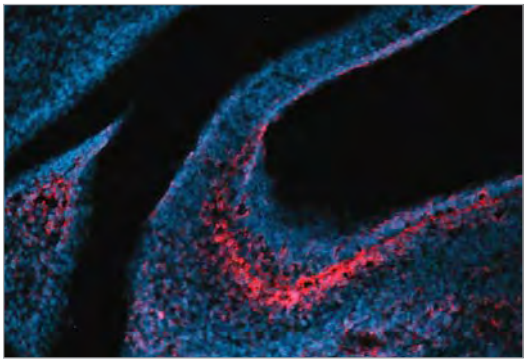
The expression of Notch and DLL4 is restricted to arterial endothelial cells early in development. Notch signaling plays a role in the specification of arterial cell identity by stimulating arterial expression of Ephrin-B2 and inhibiting EphB4 expression. Additionally, Notch signaling has been implicated in the regulation of angiogenic sprouting and branching under both physiological and pathological conditions. Several studies suggest that the DLL4-Notch pathway is required to limit angiogenic sprouting. This is accomplished by differential expression of DLL4 on endothelial tip cells and the Notch receptor on endothelial stalk cells. VEGF induces high DLL4 expression on endothelial tip cells, which activates Notch on adjacent stalk cells. Notch signaling in these cells inhibits the expression of VEGF R2 to prevent the VEGF-induced sprouting phenotype. In agreement with this model, several studies report that inhibition of DLL4-Notch signaling stimulates endothelial sprouting by allowing the endothelial tip cell phenotype to be conferred upon more cells. Despite these reports, inhibitors of DLL4-Notch signaling are still of great interest as anti-cancer agents due to the paradoxical finding that inhibition of DLL4 or Notch in mouse tumors inhibits tumor growth. Although this inhibition is accompanied by an increase in the tumor vasculature, the vessels are abnormal and nonfunctional leading to an increase in tumor hypoxia.



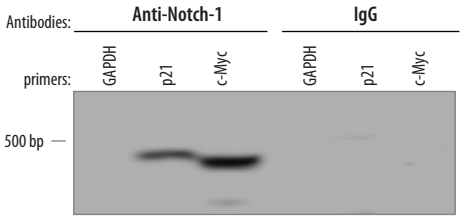
DLL4-Notch Signaling Inhibits the Tip Cell Phenotype in Endothelial Stalk Cells. Endothelial tip cells are more proliferative and have a different gene expression profile than endothelial stalk cells. The selection of an endothelial tip cell is regulated by VEGF and DLL4-Notch signaling. VEGF induces the expression of DLL4 on endothelial tip cells, which binds to Notch on adjacent stalk cells. Notch activation inhibits the expression of VEGF R2, rendering these cells less responsive to VEGF signaling. This reduced VEGF responsiveness inhibits the tip cell phenotype.



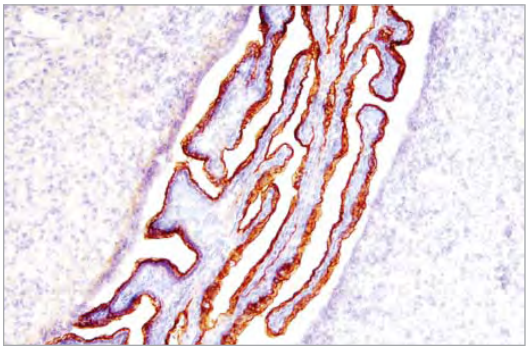
Detection of Notch-1 by Flow Cytometry. U2OS human osteosarcoma cells were stained with PE-conjugated anti-human Notch-1 (Catalog # FAB5317P; filled histogram) or PE-conjugated mouse IgG_1 isotype control (Catalog # IC002P; open histogram).



DLL1 Expression in Mouse Embryonic Stomach. DLL1 was detected in cryostat tissue sections of mouse E13.5 embryonic stomach using anti-rat DLL1 polyclonal antibody (Catalog # AF3970). Tissues were stained using NorthernLights™ 557-conjugated anti-sheep secondary antibody (Catalog # NL010; red) and counterstained with DAPI (blue).



Detection of the Notch-1 Intracellular Domain (ICD) Binding to the *p21* and *c-Myc* Promoters by Chromatin Immunoprecipitation. Human Jurkat T cells were stimulated with PMA/ionomycin, fixed, and lysed. Notch-1 binding to the *p21*, *c-Myc*, and *GAPDH* promoters was assessed using the human Notch-1 ExactaChIP™ Chromatin IP Kit (Catalog # ECP3647). Briefly, cell lysates were incubated with a biotinylated anti-human Notch-1 ICD polyclonal antibody or biotinylated anti-sheep IgG (both provided in the kit) followed by MagCellect™ Streptavidin Ferrofluid (Catalog # MAG999). DNA was purified from the immunoprecipitates and standard PCR was used to detect Notch-1 binding to the promoters of two known target genes, *p21* and *c-Myc*. Primers targeting the *GAPDH* gene were used as a negative control.



Notch-2 Expression in Rat Brain. Notch-2 was detected in a cryostat tissue section of rat brain choroid plexus using anti-rat Notch-2 polyclonal antibody (Catalog # AF1190). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

PRODUCTS FOR DELTA/NOTCH FAMILY RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	CHROMATIN IP ASSAYS
ADAM10	H M	H M		
TACE/ADAM17	H M	H	H	
CBP		H M R		
CSL		H		
DLL1	H M	H M R	H	
DLL3		H		
DLL4	H M	M	M	
DNER		H M		
HES-1		H		
HES-4		H		
Jagged 1	H R	H R		
Jagged 2	H M	H		

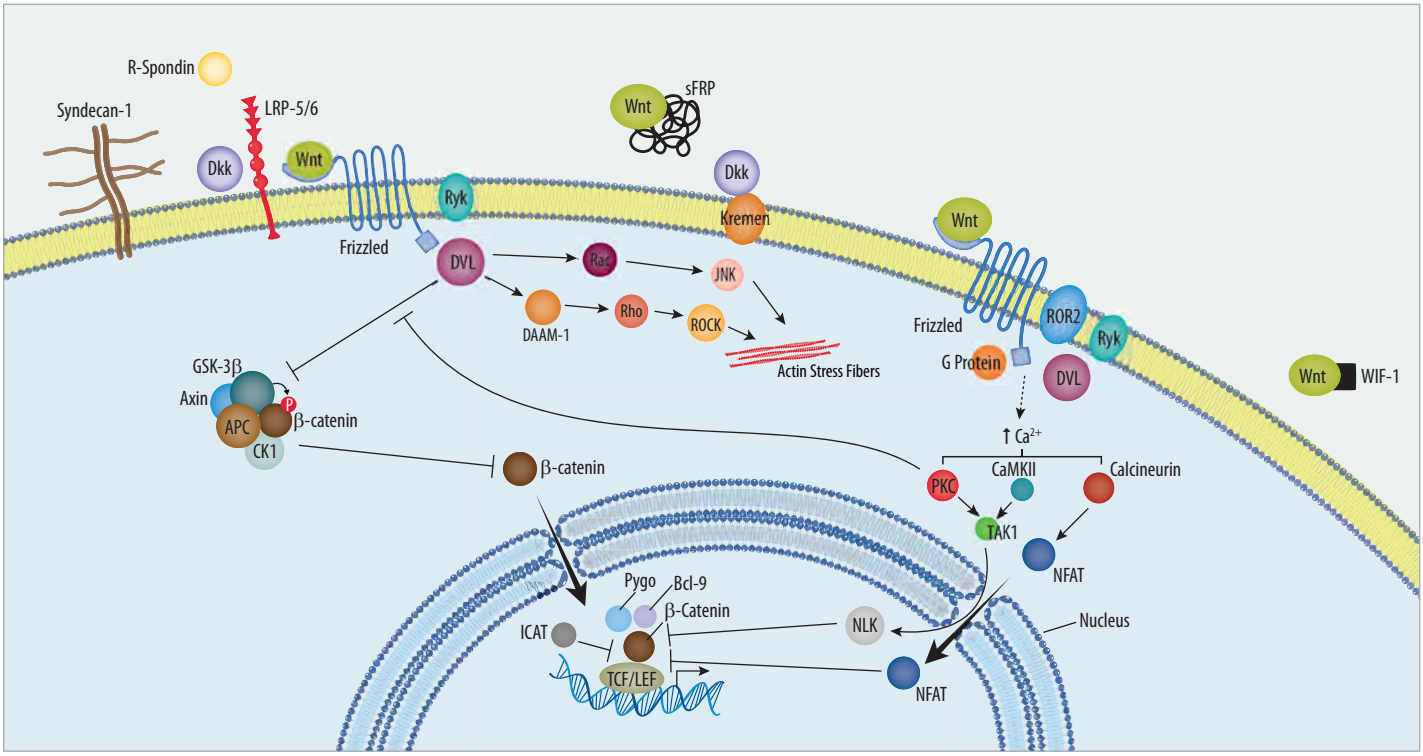
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	CHROMATIN IP ASSAYS
MAGP-1		M		
MAGP-2		H		
Nicastrin		H		
Notch-1	H M R	H M R		H
Notch-2	H M R	H R		
Notch-3	H M	H M		
Notch-4		H		
NRARP		H M		
Numb		H	H M R	
Presenilin-1		H	H	
Presenilin-2		H		
TSK	H			

For more information on products related to the Delta/Notch Family, please visit our website at www.RnDSystems.com/go/DSLNotch

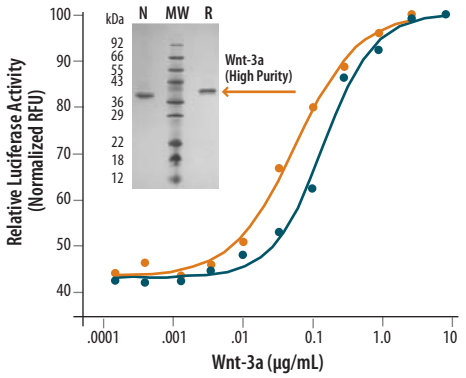
IV. Wnt Family Molecules & Modulators

Wnts are a family of cysteine-rich, secreted glycoproteins that bind to transmembrane G protein-coupled receptors belonging to the Frizzled family. Frizzled proteins form co-receptor complexes with LRP, ROR, and Ryk molecules. There are three established Wnt signaling pathways: the canonical pathway involving β-Catenin-induced transcriptional activation, the planar cell polarity pathway, and the Wnt-Ca^{2+} pathway. Activation of a given Wnt/Frizzled signaling cascade is dependent on the Wnt ligand and the cellular context.

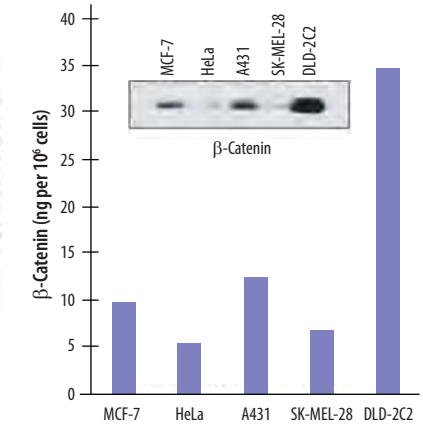
Wnt signaling has a central role in embryonic development, cell proliferation, and adult tissue homeostasis. Several studies now suggest that Wnt signaling is required for developmental angiogenesis in the placenta, ovaries, and retina. Mice deficient for Wnt-2 or Frizzled-5 have defects in vessel development in the placenta, and those lacking Frizzled-4 are unable to properly form the angiogenic corpus luteum in the ovary. This prevents embryo implantation and leads to infertility in Frizzled-4-deficient mice. In the eye, Wnt/β-Catenin signaling is induced by Norrin, which binds to Frizzled-4/LRP-5 to promote vascular development. Mutations in Norrin or Frizzled-4 both lead to defects in retinal angiogenesis. The role of the Wnt family of proteins in developmental angiogenesis in other tissues and in tumor-related angiogenesis is still unclear. Similarly, it is also unclear whether the vascular defects associated with a loss of Wnt signaling are direct or indirect effects as several angiogenic regulators are induced by Wnt signaling including VEGF, IL-8, FGFs, MMPs, Endothelin-1, uPAR, Ephrins, and Eph receptors.



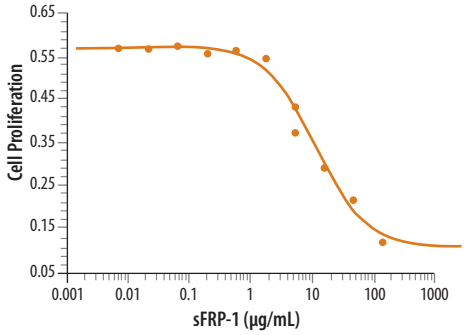
Wnt Signaling Pathways. Wnt signaling occurs through three established pathways: the canonical β-Catenin-dependent pathway, and two non-canonical β-Catenin-independent pathways, the Wnt/Ca^{2+} pathway and the planar cell polarity pathway. In the β-Catenin-dependent pathway, Wnt binding to the Frizzled receptor inactivates GSK-3β, leading to β-Catenin accumulation and gene activation. In the Wnt/Ca^{2+} pathway, Wnt binding to Frizzled results in the activation of heterotrimeric G protein-coupled receptors with subsequent activation of phospholipase C and phosphodiesterase. This results in a decrease in cGMP, an increase in intracellular Ca^{2+}, and activation of protein kinase C. In the planar cell polarity pathway, Wnt binding to Frizzled activates Dishevelled (Dsh), which then recruits RhoA/Rac, leading to JNK and ROCK activation.



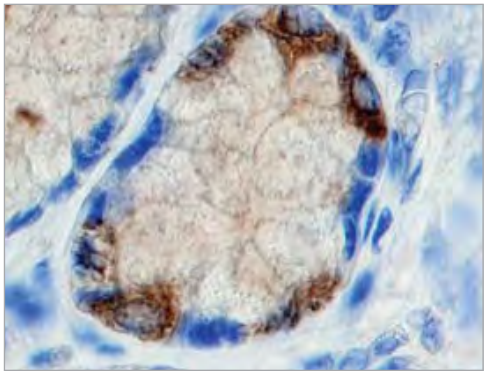
Human Wnt-3a Induces β-Catenin-responsive Transcriptional Activation. Recombinant human Wnt-3a (Catalog # 5036-WN; blue) and recombinant human Wnt-3a (High Purity; Catalog # 5036-WNP; orange) both stimulate β-Catenin-responsive transcriptional activation assessed using the TOPflash TCF reporter in the human kidney cell line, HEK293T. Purity is highlighted in a silver-stained, SDS-PAGE gel loaded with 1 µg/lane Wnt-3a (High Purity) under both non-reducing (N) and reducing (R) conditions (inset). MW = Molecular weight markers



Detection of Intracellular β-Catenin. Lysates prepared from human MCF-7, HeLa, A431, SK-MEL-28, and DLD-2C2 cancer cell lines were quantified with the human Total β-Catenin DuoSet IC ELISA (Catalog # DYC1329). The same cell lysates were also immunoblotted (inset) with anti-β-Catenin monoclonal antibody (Catalog # MAB1329). The DuoSet IC ELISA results compare well with the total amount of β-Catenin detected by Western blot.



sFRP-mediated Inhibition of HeLa Cell Proliferation. HeLa human cervical epithelial carcinoma cells were treated with increasing concentrations of His-tagged recombinant human sFRP-1(Catalog # 1384-SF) for three days. Relative cell number was determined using a MTT Cell Viability Assay (Catalog # 4890-025-K).



Detection of Wnt-2 in Human Stomach Cancer. Wnt-2 was detected in paraffin-embedded human stomach cancer sections using anti-human Wnt-2 polyclonal antibody (Catalog # AF3464). The tissue was subjected to antigen retrieval using the Basic Antigen Retrieval Reagent (Catalog # CTS013). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

PRODUCTS FOR WNT FAMILY RESEARCH LISTED ON PAGE 12>>>

PRODUCTS FOR WNT FAMILY RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	CHROMATIN IP ASSAYS
APC		H		
Axin-1		H M R		
β-Catenin		H M R X	H	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		
LRP-6	H M	H M		
Norrin	H M	H M		
Pygopus-1		M		

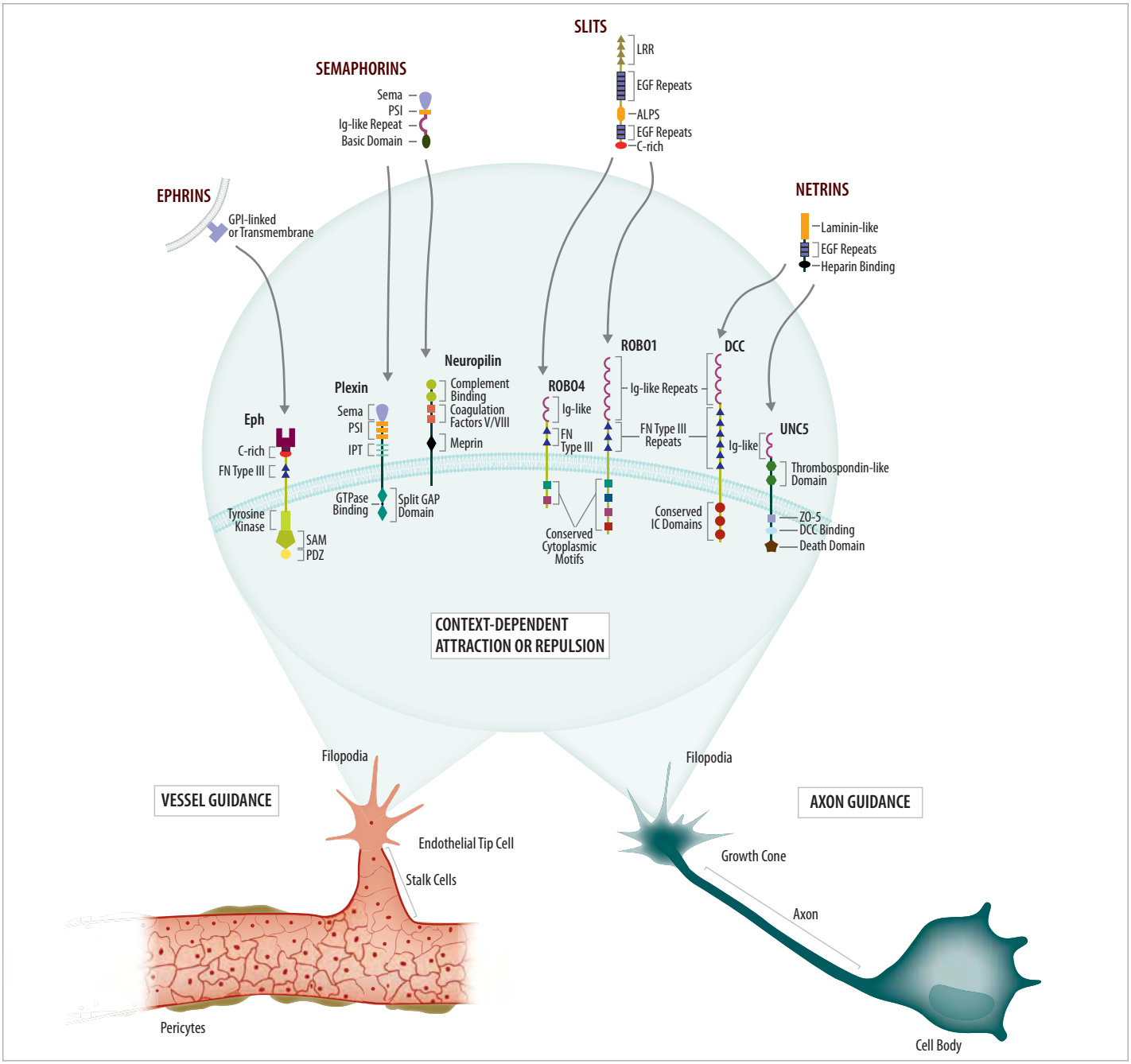
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	CHROMATIN IP ASSAYS
Pygopus-2		H		
ROR1 Receptor Tyrosine Kinase		H		
RTK-like Orphan Receptor 2/ ROR2		H		
R-Spondin 3	H	H M		
Ryk		H M		
sFRP-1*	H	H		
TCF-7/TCF-1		H		
Wnt-2		H		
Wnt-2b		M		
Wnt-3a	H M	M		
Wnt-5a	M	M		
Wnt-7a	H	H		
Wnt-10b	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

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

For more information on products for Wnt Family research, please visit our website at www.RnDSystems.com/go/Wnt

V. Guidance of Vessel Branching

It has recently been discovered that formation of the vascular system may occur by mechanisms similar to those involved in nervous system patterning. Not only do nerves and blood vessels seem to follow parallel paths, but endothelial tip cells at the ends of sprouting vessels resemble growth cones at the tips of extending axons. In addition, endothelial cells, like neurons, express receptors for classic axon guidance molecules such as Ephrins, Semaphorins, Netrins, and Slits. Due to the suggested similarities between axon and vessel guidance, the effects of these molecules on vascular development are now being investigated.

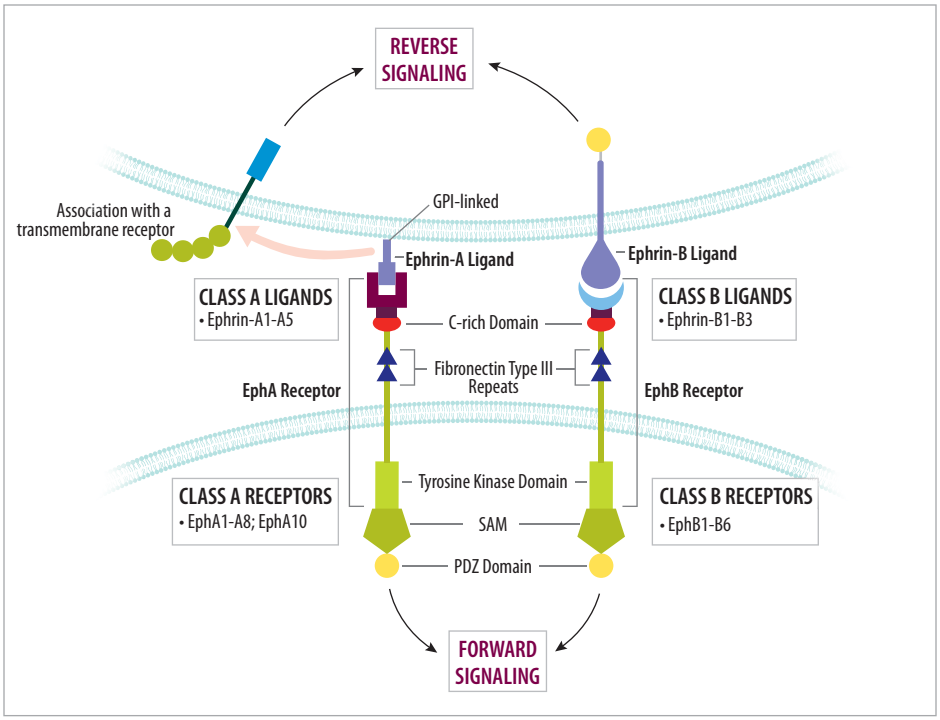


Ligand-Receptor Interactions Involved in Mediating Axon Guidance May Also Be Involved in Vascular Patterning. Axons are guided by growth cones that extend filopodia to detect attractive or repulsive signals in the extracellular environment. Ephrins, Netrins, Semaphorins, and Slits act as axon guidance signals. Integration of the signals from these molecules binding to their respective receptors on growth cones directs nervous system patterning. These same molecules have also been speculated to be involved in blood vessel sprouting and guidance. Like neurons, endothelial cells express receptors for these signaling molecules, and endothelial tip cells appear to be directed in a manner analogous to neuronal growth cones. These similarities suggest that vascular patterning may be mediated by the same mechanisms that regulate nervous system patterning.

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, where the strength of activation is dependent on receptor aggregation. In the developing nervous system, Ephrin-Eph signaling mediates axon guidance by providing repulsive cues for migrating cells and processes causing them to retreat or undergo redirection.

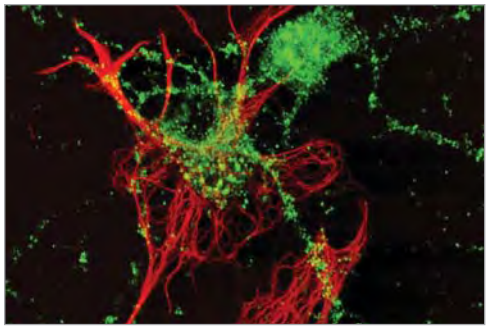
In the vascular system, Ephrin-Eph signaling is essential not only for embryonic angiogenesis, but also for neovascularization in adult organisms. Mice deficient for either Ephrin-B2 or EphB4 are embryonic lethal with defects in angiogenic branching and sprouting indicating that Ephrin-B2-EphB4 signaling is required for vascular morphorgenesis and remodeling. Ephrin-Eph signaling establishes arterio-venous cell identity during embryonic development. Ephrin-B2 is expressed by arterial endothelial cells in a VEGF-, Notch-dependent manner, while its receptor, EphB4 is expressed by venous endothelial cells. This differential pattern of expression regulates the organization of artery/vein vessel networks. Eph receptors also affect cell motility and adhesion by promoting or inhibiting Integrin functions, and may regulate adhesion to the extracellular matrix and cytoskeletal reorganization. Misregulation of Ephrin-Eph signaling contributes to the metastatic potential of some tumors likely due to cell adhesion defects. Both overexpression and underexpression of Ephrin-Eph family members correlate with a poor prognosis in several different forms of cancer.



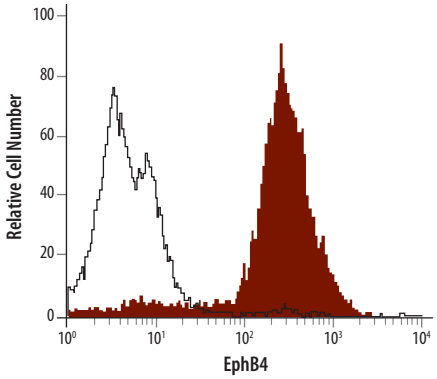
Ephrins Bind to Eph Receptors and Promote Bidirectional Signaling. Ephrins are cell surface-associated proteins that bind to Eph receptors on adjacent cells. Ephrin-A proteins are anchored to the cell via a glycosylphosphatidylinositol (GPI) linkage, while Ephrin-B ligands are transmembrane proteins. EphA receptors (EphA1-A8; EphA10) primarily bind to Ephrin-A ligands (Ephrin-A1-A5), while EphB receptors (EphB1-B6) primarily bind to Ephrin-B ligands (Ephrin-B1-B3). Ephrin-Eph interactions stimulate bidirectional intracellular signaling pathways in both ligand- and receptor-expressing cells. Since Ephrin-A ligands lack an intracellular domain, reverse signaling in these cells depends on the association of the Ephrin-A ligand with a transmembrane receptor.

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

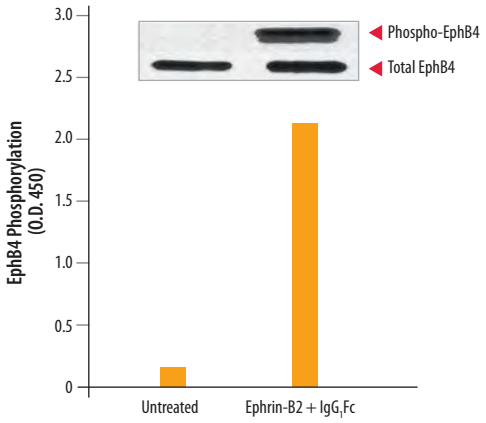
Ligand Binding Specificities for Eph Family Receptors. The ligand binding specificities for different Eph receptors are shown. [The information in the table was adapted from Surawska, H. *et al.* (2004) Cytokine Growth Factor Rev. **15**:419.]



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).



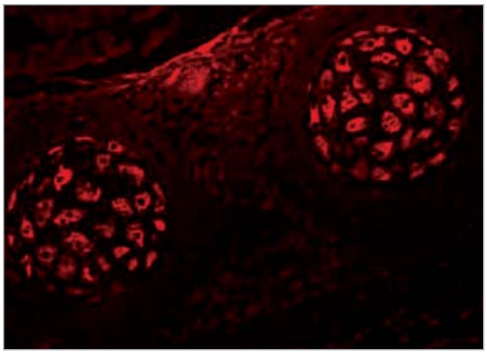
Detection of EphB4 by Flow Cytometry. MCF-7 human breast adenocarcinoma cells were stained with PE-conjugated anti-human EphB4 monoclonal antibody (Catalog # FAB3038P; filled histogram) or with PE-conjugated rat IgG_1 isotype control antibody (Catalog # IC005P; open histogram).



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_1 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) or by IP/Western blot analysis (inset). IP/Western blots were performed using anti-human EphB4 polyclonal antibody (Catalog # AF3038) and protein G agarose for immunoprecipitation, followed by immunoblotting with HRP-conjugated anti-Phospho-Tyrosine monoclonal antibody (Catalog # HAM1676) to detect phospho-EphB4. Membranes were stripped and total EphB4 was detected using biotinylated anti-human EphB4 polyclonal antibody (Catalog # BAF3038).

PRODUCTS FOR EPHRIN-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL 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	



EphA4 Expression in Rat Cartilage. EphA4 was detected in a cross-section of rib cartilage primordium in E15 rat embryos using anti-mouse EphA4 polyclonal antibody (Catalog # AF641).

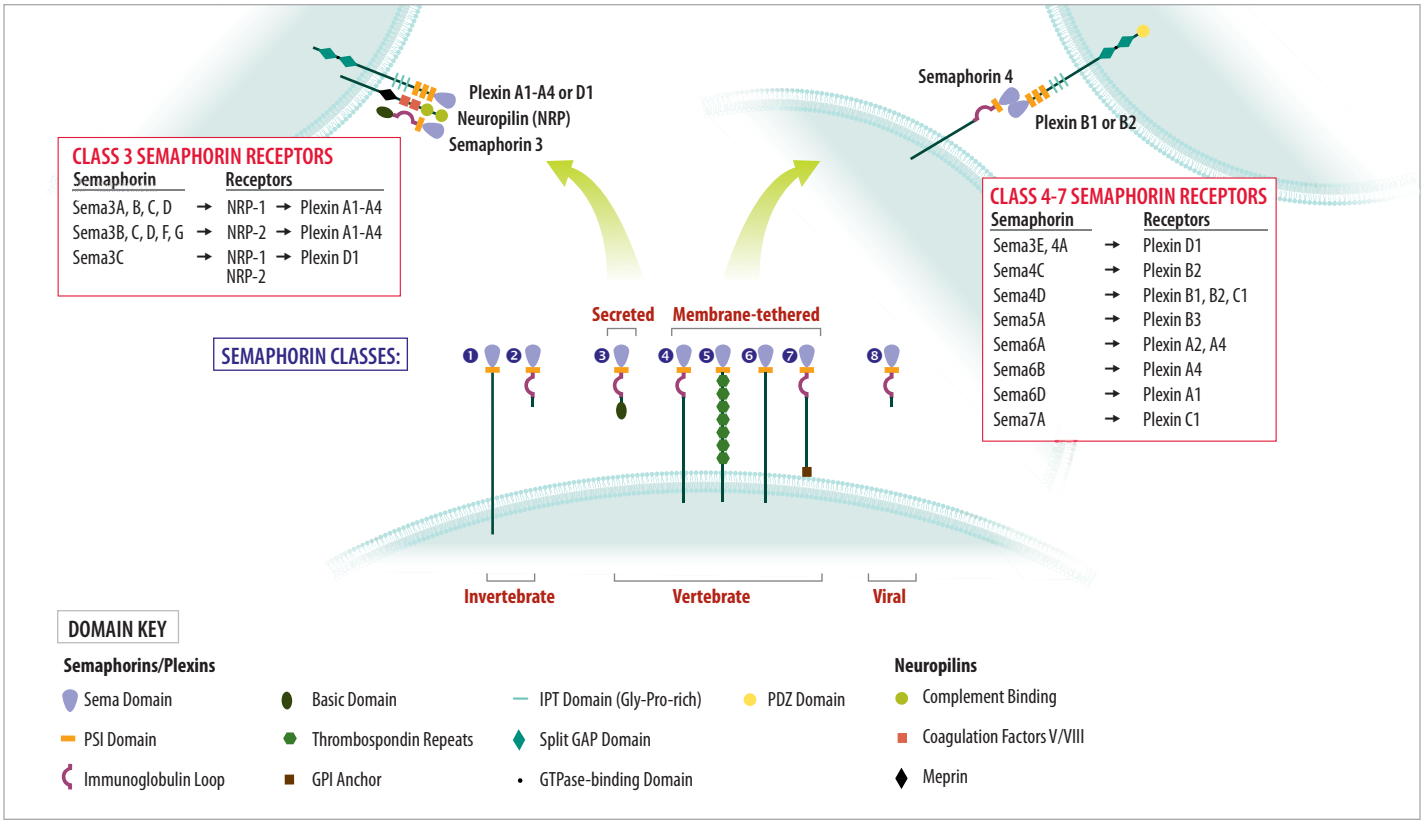
For more information on Ephrin-Eph-related products, please visit our website at www.RnDSystems.com/go/Ephrins

Semaphorins, Neuropilins, & Plexins

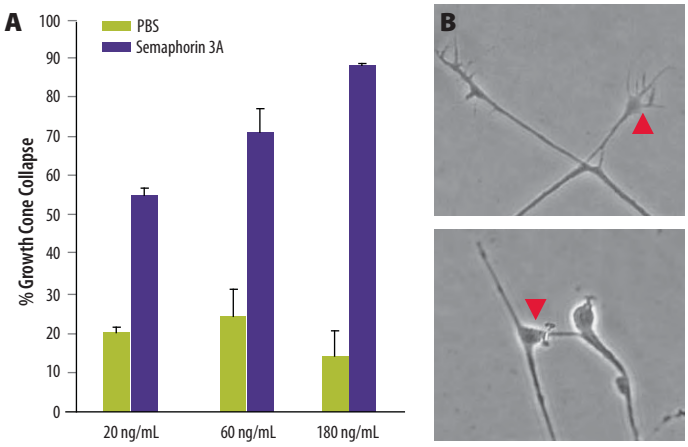
The Semaphorins constitute a large family of secreted and membrane-tethered signaling molecules that can be divided into eight classes according to their structure and species of origin. Classes 1 and 2 are found in invertebrates, classes 3 through 7 are found in vertebrates, and the final class is viral. 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 (and also serve as co-receptors for some forms of VEGF), 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.

Neurons and endothelial cells are two cell types on which Neuropilins and Plexins are highly expressed. 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*. In the vascular system, Semaphorin-Plexin signaling is responsible for regulating endothelial cell migration and vascular remodeling, as well as vascular exclusion from the somites. While Semaphorin-3A and -3F inhibit angiogenesis, other Semaphorins such as Semaphorin-4D and -6D may act as pro-angiogenic factors. Semaphorin-3B and -3F, which are inactivated in small cell lung carcinomas, have been suggested to function as tumor suppressor proteins.

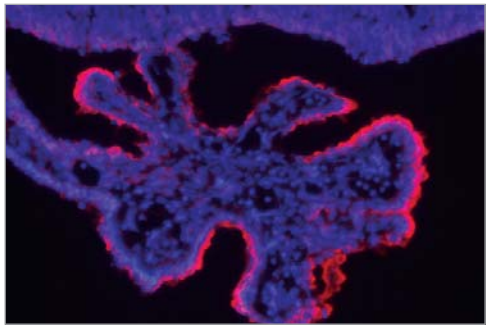
Neuropilins also play a role in vascular development. Mice overexpressing Neuropilin-1 (NRP-1) and NRP-1-deficient mice both display severe nervous and vascular system defects and are embryonic lethal. The vascular defects observed in these mice are presumably due to misregulation of VEGF-VEGF R2 signaling during developmental angiogenesis. In contrast, Neuropilin-2 (NRP-2)-deficient mice develop blood vessels normally, but have defects in lymphatic vessel growth suggesting that NRP-2 is involved in regulating VEGF-C-mediated lymphangiogenesis. High levels of NRP-1 and NRP-2 expression have been found in multiple different types of tumors, and inhibitors of NRP-1 have been shown to prevent vascular remodeling and inhibit tumor growth. In addition to mediating VEGF and Semaphorin/Plexin signaling, NRP-1 and NRP-2 interact with a variety of other ligands and receptors including HGF/c-MET, and PDGF-B.



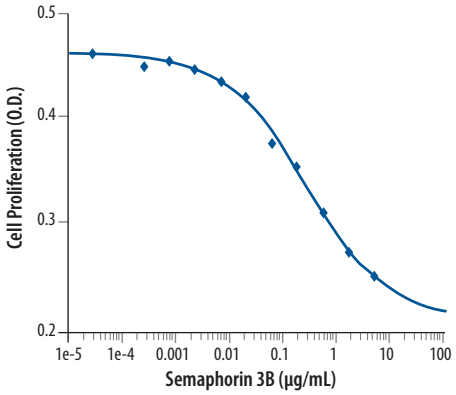
Multiple Receptors or Receptor Complexes Mediate Semaphorin Signaling. Semaphorins are divided into eight classes: Classes 1 and 2 are found in invertebrates, classes 3-7 are found in vertebrates, and class 8 is viral. Of the vertebrate Semaphorins, Class 3 Semaphorins are secreted and require both Neuropilins (NRP-1 and NRP-2) and Plexins for signaling. Class 4-7 Semaphorins are membrane-tethered and can activate Plexins directly to initiate signaling. Specific Semaphorin 3-Neuropilin-Plexin complexes and Semaphorin 4-7-Plexin interactions are shown in the tables.



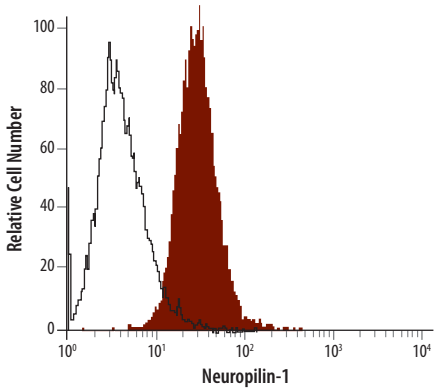
Semaphorin 3A-induced Growth Cone Collapse. A. E8 chick dorsal root ganglion explants, grown in the presence of recombinant human β-NGF (Catalog # 256-GF), were incubated with PBS or with increasing concentrations of recombinant human Semaphorin 3A (Catalog # 1250-S3). The percent of growth cone collapse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



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



Semaphorin 3B-induced Inhibition of A549 Cell Proliferation. A549 human lung carcinoma cells were incubated with increasing concentrations of recombinant mouse Semaphorin 3B (Catalog # 5440-S3). Relative cell number was determined in a colorimetric assay using the tetrazolium salt, MTT (Catalog # 4890-025-K).



Detection of Neuropilin-1 by Flow Cytometry. Human umbilical vein endothelial cells (HUVECs) were stained with PE-conjugated anti-human Neuropilin-1 monoclonal antibody (Catalog # FAB3870P; filled histogram) or with PE-conjugated rat IgG_{2A} isotype control (Catalog # IC002P; open histogram).

PRODUCTS FOR SEMAPHORIN, NEUROPILIN, & PLEXIN-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES
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	H
Semaphorin 3B	M	
Semaphorin 3C	H	M
Semaphorin 3E	H M	H

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES
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

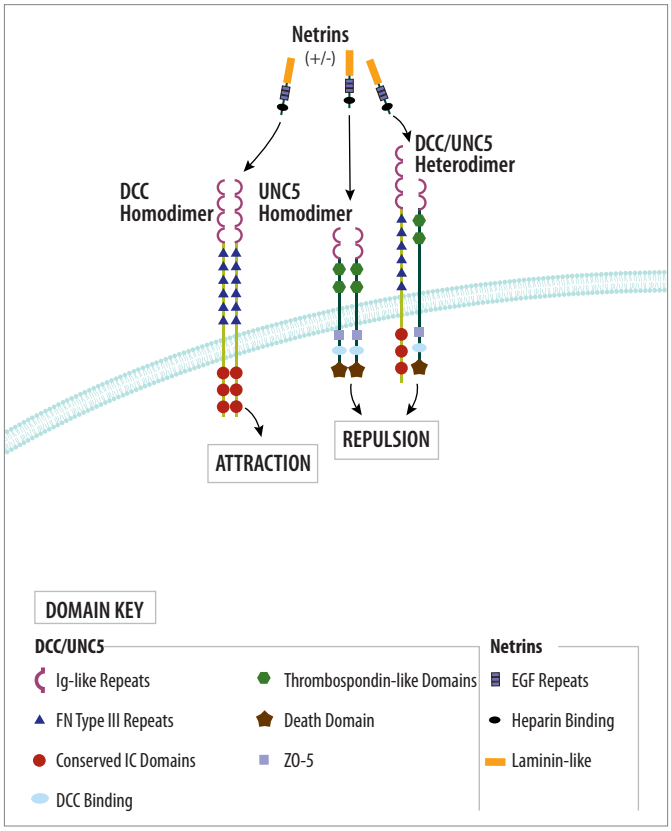
** This product is covered under one or more patents owned by the Regents of the University of California.*

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

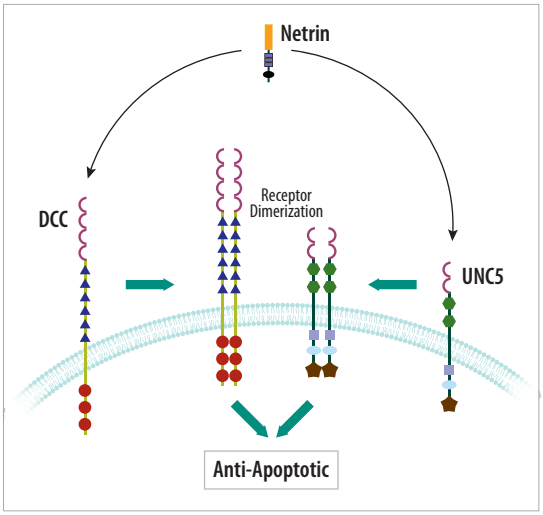
Netrins & DCC/UNC5 Receptors

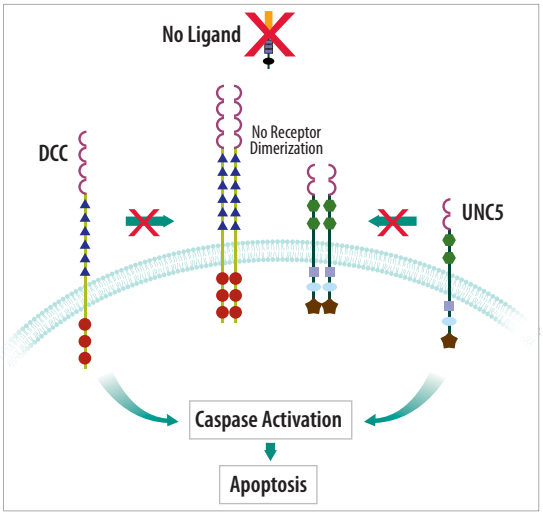
Netrins are a small family of laminin-related molecules that includes both secreted [Netrin-1, -3, and -4 (β Netrin)] and membrane-associated proteins (Netrin-G1, -G2). Netrins bind to UNC5 and DCC family receptors to mediate context-dependent repulsive (UNC5) or attractive (DCC) effects on axon guidance. UNC5 and DCC receptors form homodimers and heterodimers to regulate signaling. The role of Netrins and Netrin receptors in vascular development is not well-characterized, but several observations suggest that they contribute to the regulation of vessel sprouting or guidance. Netrin-1 has been shown to stimulate endothelial cell proliferation, migration, and tube formation *in vitro* and to promote neovascularization in an *in vivo* model of ischemia. While this suggests a pro-angiogenic function, the results of other studies indicate that Netrin-1 may inhibit angiogenesis. For example, mice lacking UNC5B, a Netrin-1 receptor predominantly expressed in the vasculature, display excessive capillary branching and are embryonic lethal. Additionally, Netrin-1-UNC5B signaling was found to promote endothelial tip cell retraction and inhibit sprouting angiogenesis. Likewise, Netrin-4 has also been reported to inhibit endothelial cell migration and tumor cell proliferation *in vitro*. Collectively, these results suggest that Netrins, like other vessel guidance cues, may have a context-dependent, bifunctional role in vascular development.

Several studies now suggest a link between Netrin receptors and the regulation of apoptosis. These studies characterize Netrin receptors as "dependence receptors", inhibiting apoptosis when bound by their ligands, but promoting apoptosis when unoccupied. This effect may be due to ligand-mediated receptor dimerization, which has been hypothesized to block a pro-apoptotic signal. High levels of Netrin-1 expression or reduced expression of its receptors has been found in certain forms of cancer including colorectal and prostate cancer. It is likely that DCC functions as a tumor suppressor by inducing apoptosis in cells that are not exposed to Netrins.



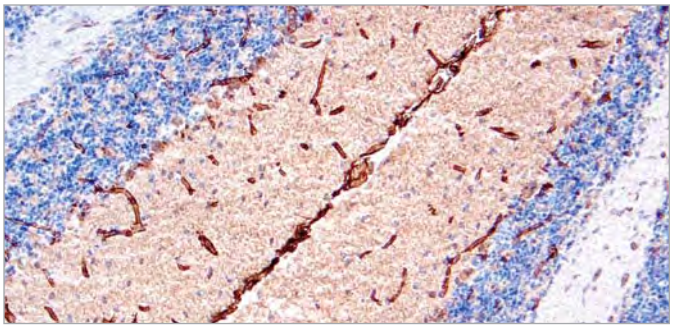
Netrins Bind to UNC5 and DCC Family Receptors. Netrins bind to both the UNC5 and DCC receptor families and mediate context-dependent repulsive or attractive effects on axon guidance. Activation of UNC5 promotes repulsion, while Netrin binding to DCC has attractive effects. Interactions between Netrins and their receptors may direct vessel guidance in an analogous manner.





Netrin Receptors Promote Apoptosis in the Absence of Netrins. DCC and UNC5 receptors have been linked to apoptosis. Ligand binding to UNC5 or DCC receptors promotes receptor dimerization that blocks a pro-apoptotic signal (left), but in the absence of ligand, the receptor fails to dimerize, exposing a pro-apoptotic signal (right).

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish



Netrin-4 Expression in Mouse Cerebellum. Netrin-4 was detected in cryostat tissue sections 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).

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

PRODUCTS FOR NETRIN-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS
DCC*	M	H M	
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
UNC5H1*	R	R	
UNC5H2*	R	R	
UNC5H3*	H	H	
UNC5H4	H	H	

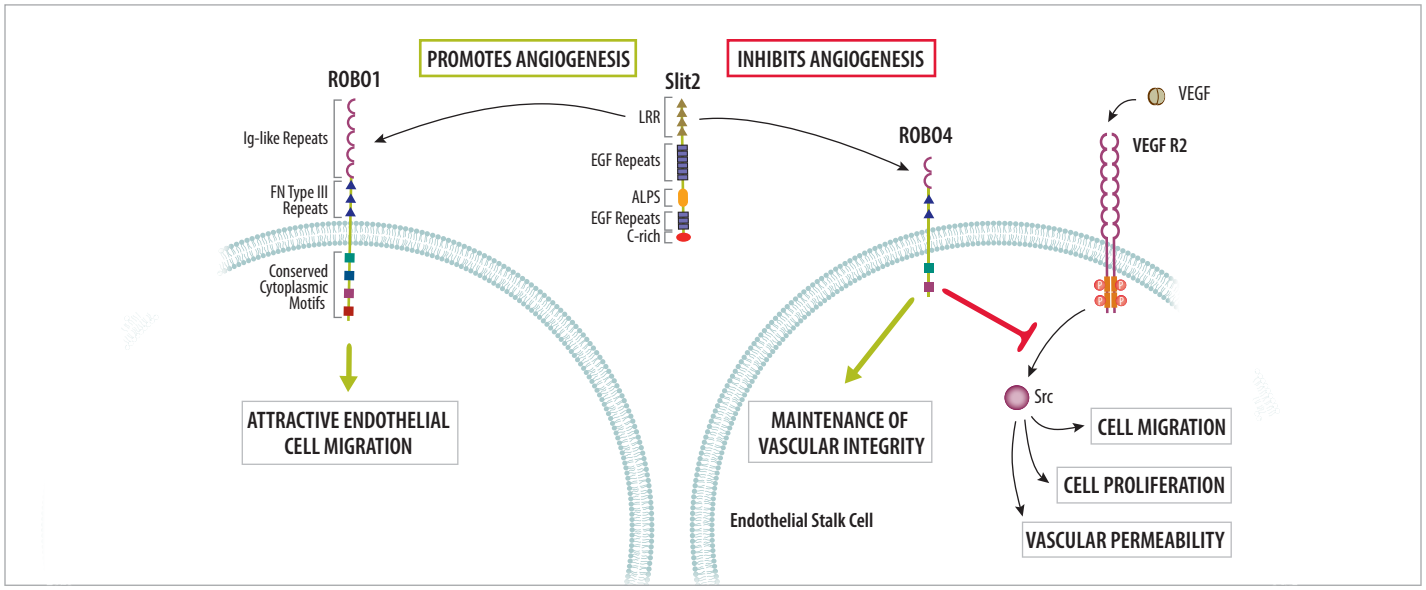
**This product is covered under one or more patents owned by the Regents of the University of California.*

Slit Proteins & ROBO Receptors

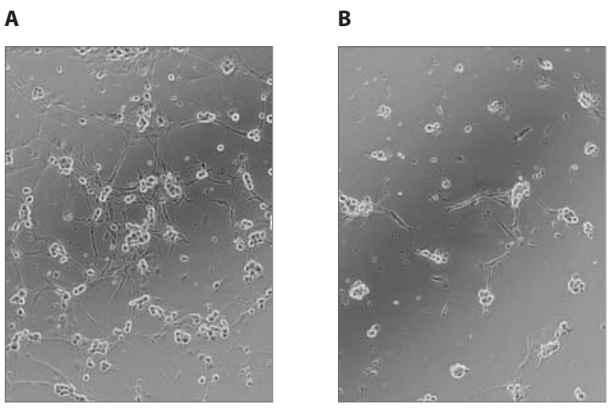
The Slit family of proteins consists of three members (Slit1-3) that signal by binding to one of four Roundabout (ROBO1-4) receptors. Slits are large, secreted glycoproteins that are subject to proteolytic cleavage resulting in fragments with variable activities. ROBO1, ROBO2, and ROBO3 are predominantly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

ROBO4 and ROBO1 are also expressed on vascular endothelial cells, but their roles in vessel guidance are controversial. Independent studies have shown that both ROBO1 and ROBO4 bind to Slit2, but they seem to mediate different effects on endothelial cell migration. While ROBO1 signaling attracts endothelial cells to promote angiogenesis, ROBO4 has been shown to inhibit endothelial cell migration. In addition, Slit2-ROBO4 signaling in endothelial stalk cells has been suggested to promote vascular integrity by inhibiting VEGF-induced migration, tube formation, and hyperpermeability.

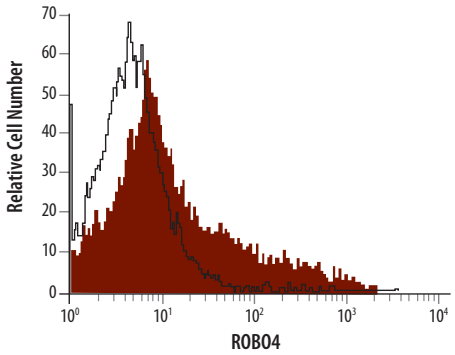
Conflicting evidence has also been reported regarding the involvement of Slit-ROBO signaling in tumor-related angiogenesis. Both Slit2 and ROBO1 have been found to be inactivated in certain cancer cell lines suggesting that they may act as tumor suppressors. In contrast, expression of Slit2 by tumor cells has been shown to attract endothelial cells and mediate tumor angiogenesis and growth through ROBO1 signaling. Further understanding of ligand-receptor interactions and ROBO receptor heterodimerization may allow this pathway to be targeted for the development of angiogenic inhibitors.



Slit-ROBO Signaling Exerts Paradoxical Effects on Endothelial Cells. Slit2 exerts seemingly paradoxical effects on endothelial cells by binding to ROBO1 or ROBO4. Slit2 binding to ROBO1 promotes endothelial cell migration, while Slit2 binding to ROBO4 inhibits the effects of VEGF-induced signaling including endothelial cell migration, proliferation, and vascular permeability. This occurs via the inhibition of Src family kinases. Inhibition of VEGF signaling suggests that Slit2-ROBO4 may be involved in maintaining vascular integrity in endothelial stalk cells.



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 µg/mL). The presence of the Slit2 protein significantly enhanced neurite outgrowth.



Detection of ROBO4 by Flow Cytometry. Human umbilical vein endothelial cells (HUVECs) were incubated with anti-human ROBO4 monoclonal antibody (Catalog # MAB24541; filled histogram) or with mouse IgG_{2B} isotype control (Catalog # MAB0041; open histogram). The cells were stained with APC-conjugated anti-mouse antibody (Catalog # F0101B).

PRODUCTS FOR SLIT FAMILY RESEARCH

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

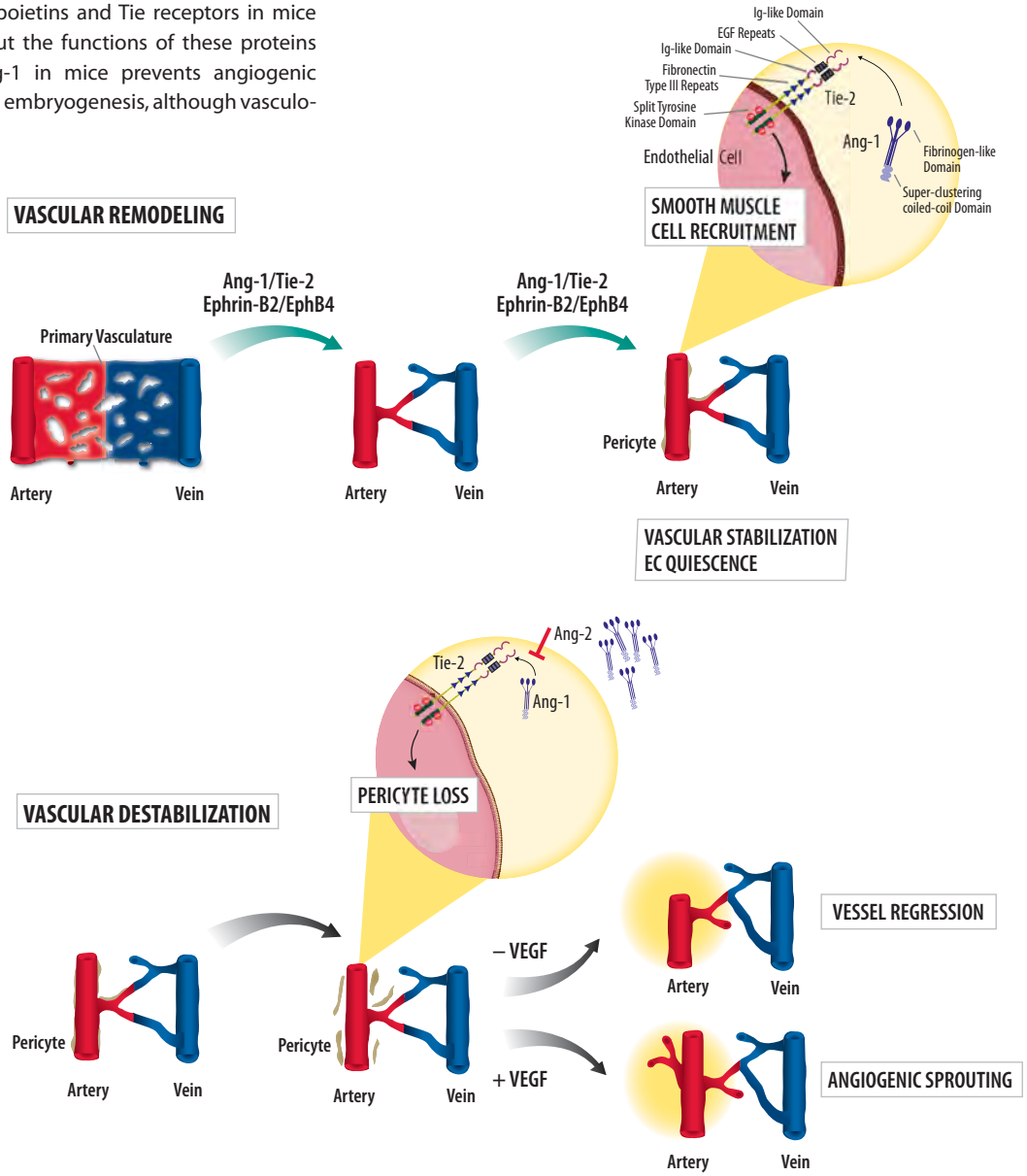
For more information on products related to the Slit or ROBO families, please visit our website at www.RnDSystems.com/go/SlitROBO

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

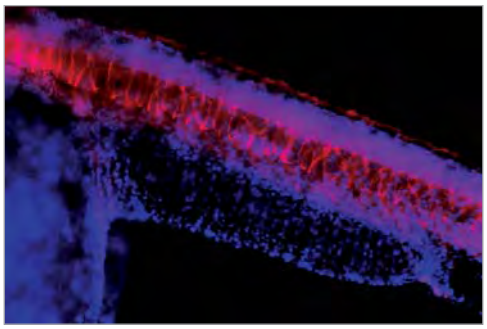
VI. Angiopoietins & Tie Receptors

Tie-1 and Tie-2 constitute a subfamily of receptor tyrosine kinases that are expressed predominantly on endothelial and early hematopoietic cells. While the ligand for Tie-1 has not been clearly established, Tie-2 serves as a receptor for the Angiopoietin family of growth factors (Ang-1, Ang-2, Ang-3, Ang-4). Angiopoietins are secreted proteins with an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain that mediates receptor binding. Angiopoietin-Tie signaling plays a critical role in endothelial cell differentiation and blood vessel morphogenesis by regulating angiogenic remodeling processes such as vessel stabilization/destabilization and pericyte recruitment or loss. Angiopoietins function in a context-dependent manner. In general, Ang-1 and Ang-4 act as agonists to promote Tie-2-dependent vascular remodeling, while Ang-2 and Ang-3 have antagonistic effects.

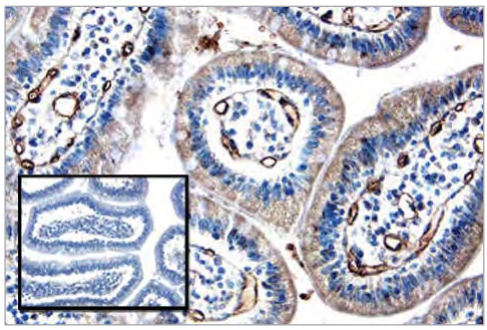
Knockout or overexpression of Angiopoietins and Tie receptors in mice has provided critical information about the functions of these proteins *in vivo*. Lack of either Tie-2 or Ang-1 in mice prevents angiogenic remodeling and causes lethality early in embryogenesis, although vasculogenesis is normal. Overexpression of Ang-2 results in the same phenotype, indicating that Ang-2 likely acts as an antagonist of Ang-1-Tie-2 signaling. In contrast, Ang-1 overexpression in the skin leads to an increase in vessel size and promotes vessel integrity, suggesting that Ang-1-Tie-2 sig-naling is involved in maintaining endothelial cell quiescence. Recent studies indicate that Tie-1 may also bind to Angiopoietins and inhibit Ang-1-Tie-2 signaling, possibly acting in a complex with Tie-2. Targeted disruption of Tie-1 results in embryonic lethality due to a loss of vessel integrity and excessive capillary sprouting. Whether the Angiopoietin-Tie pathway can be targeted to inhibit tumor-related vascular maturation or to prevent vessel leakiness in ischemic diseases is currently being investigated.



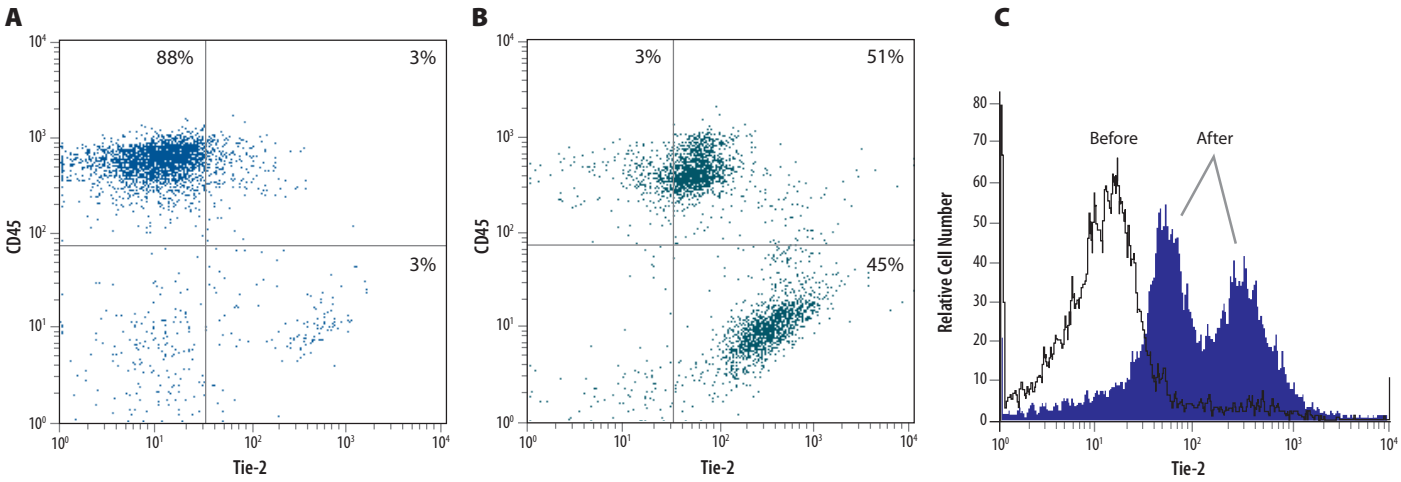
Vascular Remodeling Processes are Governed by Angiopoietin-Tie-2 Signaling. Remodeling of the primary vasculature is directed by Angiopoietin-1-Tie-2 and Ephrin-B2-EphB4 signaling pathways. Angiopoietins are secreted proteins with an N-terminal coiled-coil domain that mediates oligomerization and a C-terminal fibrinogen-like domain that mediates receptor binding. Angiopoietins bind to Tie-2 through its second extracellular Ig-like domain. Upon ligand binding, Tie-2, a receptor tyrosine kinase, becomes autophosphorylated on its split intracellular tyrosine kinase domain, leading to the activation of downstream signaling pathways that mediate vascular remodeling, vascular stabilization, and endothelial cell quiescence. Ang-1-Tie-2 signaling is required for pericyte and smooth muscle cell recruitment and for maintenance of pericyte-endothelial cell interactions that promote endothelial cell quiescence. Angiogenesis is characterized by endothelial cell activation and angiogenic sprouting, which requires Ang-2-mediated vascular destabilization. Ang-2 acts as an antagonist of Ang-1-Tie-2 signaling, promoting pericyte loss and subsequent vessel destabilization. In the presence of VEGF, this will lead to angiogenic sprouting. In the absence of VEGF, it will lead to vessel regression.



Tie-2 Expression in Developing Blood Vessels. Tie-2 was detected in developing embryonic zebrafish blood vessels using anti-zebrafish Tie-2 polyclonal antibody (Catalog # AF928) followed by NorthernLights™ 557-conjugated anti-goat IgG (Catalog # NL001; red). Tissues were counterstained with DAPI (blue).



Ang-2 Expression in Human Gastrointestinal Cancer Tissue. Ang-2 was detected in paraffin-embedded human gastrointestinal cancer tissue sections using anti-human Ang-2 polyclonal antibody (Catalog # AF623). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Inset shows control staining without primary antibody.



Isolation of Tie-2⁺ Cells Using the PlusCellect™ Kit. Peripheral blood mononuclear cells (PBMCs), depleted of monocytes using the MagCellect™ Human CD14⁺ Cell Isolation Kit (Catalog # MAGH105), were spiked with human umbilical vein endothelial cells (HUVECs). Tie-2⁺ cells were isolated from this mixture by positive selection using the Human Tie-2 PlusCellect Kit (Catalog # PLS313). Cells were stained with PE-conjugated anti-human Tie-2 monoclonal antibody (provided in the kit) before **(A)** and after **(B)** selection and with APC-conjugated anti-human CD45 monoclonal antibody (Catalog # FAB1430A) to detect contaminating blood cells. A histogram depicting data from the Tie-2⁺ staining in (A) and (B) is also shown **(C)**.

PRODUCTS FOR ANGIOPOIETIN-RELATED RESEARCH

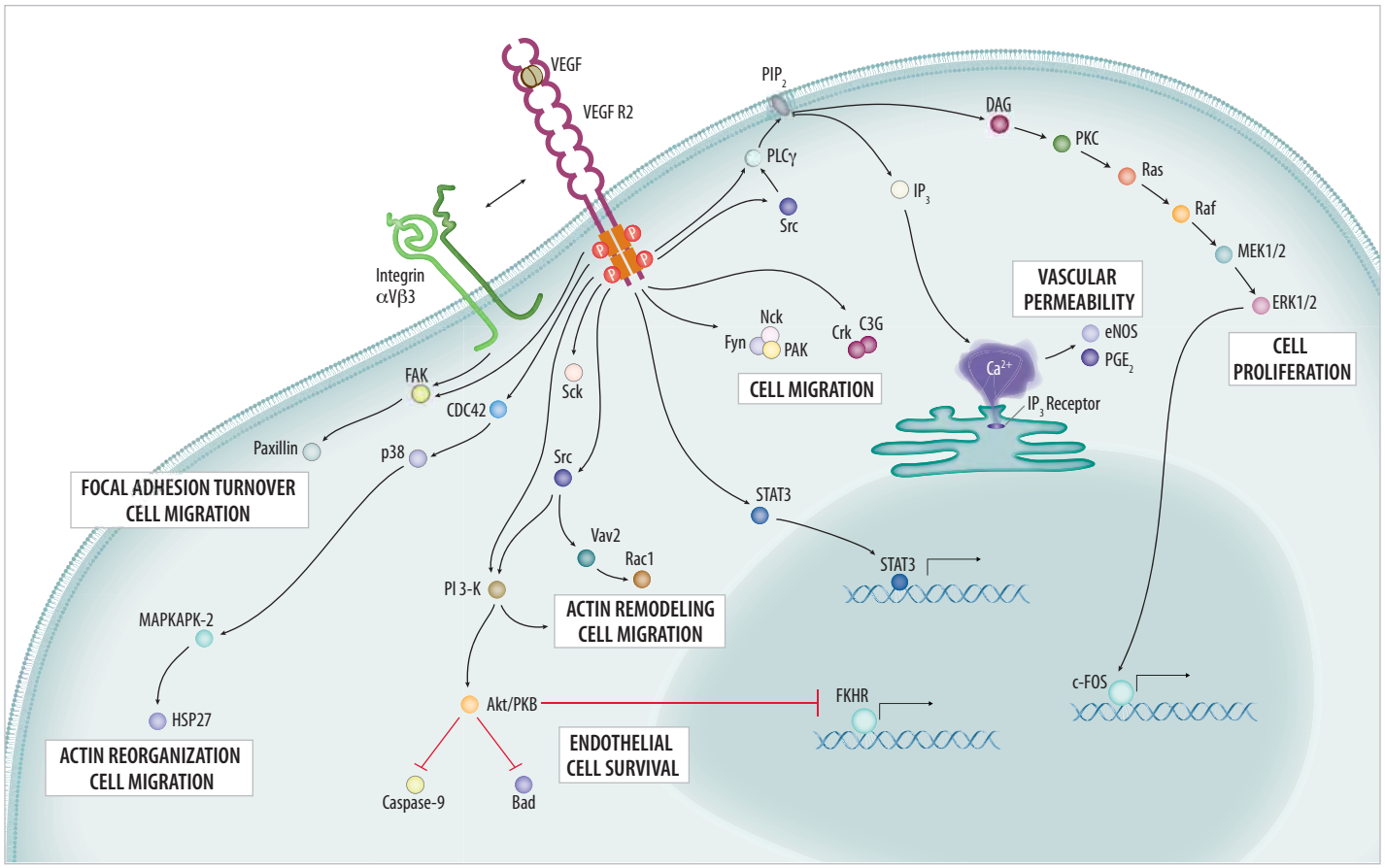
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS
Angiopoietin-1	H	H	H	H	
Angiopoietin-2	H	H	H		
Angiopoietin-3	M	M	M		
Angiopoietin-4	H	H			
Angiopoietin-like 1		H			
Angiopoietin-like 2		H M			
Angiopoietin-like 3	H M	H M	M		
Angiopoietin-like 4/ANGPTL4	H M	H	H		
Angiopoietin-like Factor/ANGPTL7		H			
Tie-1	H	H	H		
Tie-2	H M R Z	H M Z	H M		H

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

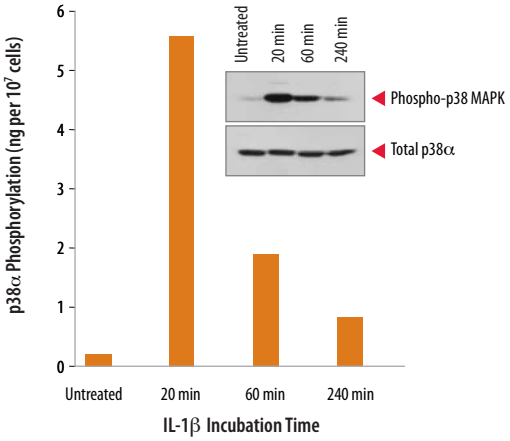
KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

VII. Intracellular Signaling Molecules Involved in Angiogenesis

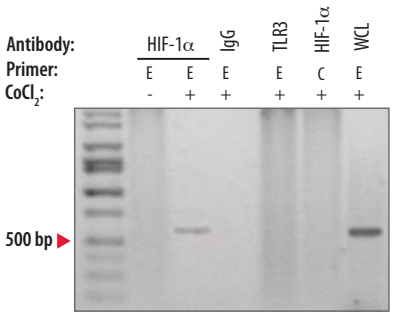
Angiogenesis is regulated by multiple ligand-receptor interactions, which activate intracellular signaling pathways that promote endothelial cell activation. For example, VEGFs and FGFs bind to tyrosine kinase receptors that dimerize upon ligand-binding and undergo autophosphorylation. Phosphorylation of these receptors leads to the recruitment of adaptor molecules and the activation of several intracellular kinases including mitogen-activated protein kinases (MAPKs), PLC-γ, PKC, PI 3-K, and Akt/PKB. Activation of these kinases leads to a cascade of phosphorylation reactions with multiple outcomes. One outcome is the transcriptional activation of target genes that promote endothelial cell survival and proliferation. Another outcome is the activation of factors that can promote cytoskeletal reorganization and cell motility. Intracellular signaling pathways direct endothelial cell activities and ultimately promote or inhibit angiogenesis.



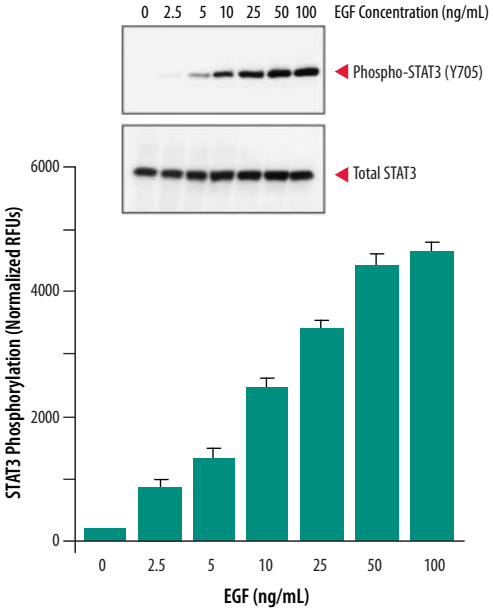
VEGF Intracellular Signaling Pathways. VEGF mediates intracellular signaling pathways by binding to one of three receptor tyrosine kinases, VEGF R1/Flt-1, VEGF R2/KDR/Flk-1, or VEGF R3/Flt-4. VEGF binding to VEGF R2 promotes signal transduction cascades that culminate in angiogenic processes such as endothelial cell proliferation, migration, and vascular permeability.



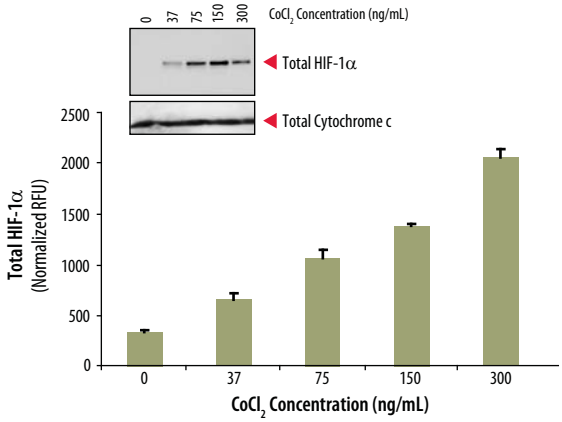
p38α Phosphorylation in IL-1β-treated HepG2 Cells. HepG2 human hepatocellular liver carcinoma cells were treated with recombinant human IL-1β (Catalog # 201-LB; 10 ng/mL) for the indicated times. Cell lysates from treated and non-treated cells were assessed for p38α phosphorylation using the human/mouse/rat Phospho-p38α (T180/Y182) Surveyor™ IC ELISA Kit (Catalog # SUV869; bar graph). The same lysates were also immunoblotted using anti-human/mouse/rat Phospho-p38 MAPK (T180/Y182) polyclonal antibody (Catalog # AF869) and anti-human/mouse/rat total p38α monoclonal antibody (Catalog # MAB869; inset).



Detection of HIF-1α Binding to the Epo Promoter by Chromatin Immunoprecipitation. Mouse primary kidney cells, untreated (-) or treated with the hypoxia mimetic $CoCl_2$ for 16 hours (+), were fixed and lysed. HIF-1α binding to the *Epo* promoter (E), or the *Collagen I* promoter (C) as a negative control, was assessed using the HIF-1α Exact-aChIP™ Chromatin IP Kit (Catalog # ECP1935). Briefly, cell lysates were incubated with biotinylated anti-human/mouse HIF-1α polyclonal antibody, or as negative controls, with biotinylated anti-human TLR3, or with biotinylated anti-goat IgG polyclonal antibodies, followed by MagCellect™ Streptavidin Ferrofluid (Catalog # MAG999). DNA was purified from the immunoprecipitates and binding to the *Epo* or *Collagen I* promoters was detected by standard PCR. Lane 1 = DNA marker, WCL = PCR positive control using an aliquot of the whole cell lysate.



EGF-induced STAT3 (Y705) Phosphorylation Assessed using the Cell-Based ELISA. A431 human epidermoid carcinoma cells were treated with the indicated concentrations of recombinant human EGF (Catalog # 236-EG) for 10 min. Phosphorylation of STAT3 on Y705 was determined using the human/mouse Phospho-STAT3 (Y705) Cell-Based ELISA (Catalog # KCB4607) and normalized to total STAT3 in the same wells (bar graph). Values represent the mean ± the range of duplicate determinations. Detection of STAT3 (Y705) phosphorylation by Western blot using the antibodies provided in the ELISA kit is shown for comparison (inset).



Measurement of HIF-1α protein levels in MCF-7 cells using the Total HIF-1α Cell-Based ELISA Kit. MCF-7 human breast adenocarcinoma cells were seeded at approximately 1.5 x 10^4 cells per well in 96-well plates 16 hours before treatment. Cells were treated with the indicated amounts of $CoCl_2$, a hypoxia mimetic, for 8 hours. After fixation of cells in the wells, HIF-1α levels were determined and normalized to total cytochrome c (a housekeeping protein) in the same well using the Total HIF-1α Cell-Based ELISA Kit (Catalog # KCB1935). Values represent the mean ± the range of duplicate determinations. Western blot analysis of total HIF-1α and total cytochrome c using the antibodies supplied in this Cell-Based ELISA kit is also shown (inset).

PRODUCTS FOR INTRACELLULAR SIGNALING RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	TRANSCRIPTION FACTOR BINDING & CHROMATIN IP ASSAYS
Akt		H M R	H M R	
Akt1	H	H M R	H M R	
Akt2		H M R		
Akt3		H		
ARNT		H		
CaM Kinase II		H M R X		
Crk		H		
ERK1/ERK2		H M R	H M R	
ERK1	H	H M R	H M R	
ERK2	H	H M R	H M R	
FAK	H	H M R		
Fyn		H M R		
GRB2		H M R		
GSK-3α/β		H M R	H M R	
GSK-3α		H M R	H	
GSK-3β	H	H M R	H M R	
HIF-1α		H M R	H M	H M
HSP27	H	H M R	H M R	
Jak1		H M R		
Jak2		M R		
Jak3		H		
JNK		H M R	H M R	
JNK2		H M R	H M R	
JunB		H		
LKB1		H		
MAPKAPK2	H			
MEK1/MEK2		H M R	H M R	
MEK1		H M R B Ca Ch Pr X		
MEK2	H	H M R		
NFκB1		H M		H
NFκB2		H		H
p38α	H	H M R	H M R	
p38γ		H M R	H M R	
p38δ		H	H	
p70 S6 Kinase	H	H M R	H M R	

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	TRANSCRIPTION FACTOR BINDING & CHROMATIN IP ASSAYS
p300		H		H
p38 MAP Kinase		H M R	H M	
Paxillin		H M R	H M R	
PDK-1	H	H		
PI 3-Kinase p85α		H M R		
PI 3-Kinase p110β		H		
PI 3-Kinase p110δ		H		
PI 3-Kinase p110γ		H		
PKCα		H M R		
PKCβ1		H R		
PKCβ2		H M		
PKCδ	H			
PKCε		H M R		
PKCγ		H M R		
PKCι/λ		H M R		
PKCθ		H M		
PTEN	H	H M R	H M R	
Raf-1	H	B Ca Ch Pr		
Ras		H M R		
Ras-GAP		H M R		
ROCK1	H			
ROCK2		H M R		
SCL/Tal1		H		
Smad1		H		
Smad2		H M		H
Smad2/3		H M		H
Smad3		H M		H
Smad4		H		H
Smad5		H		
Smad7		H M R		
Smad8		H		
Src	H V	H M R	H	
STAT1		H M		H M
STAT3		H M R	H M	H M
STAT5a/b		H M	H M	H M

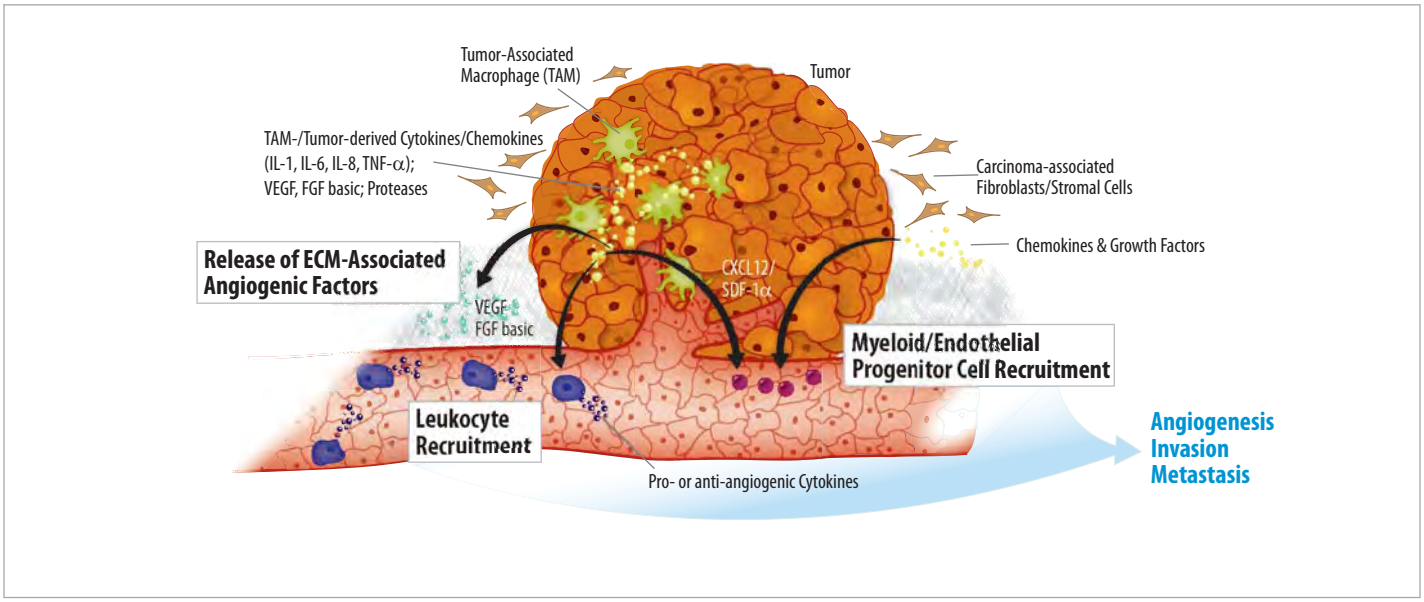
For more information on intracellular signaling molecules, please visit our website at www.RnDSystems.com/go/Signaltransduction

VIII. Secreted Signaling Molecules & Angiogenesis

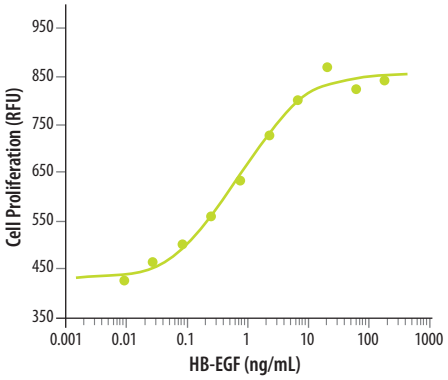
Cytokines & Receptors

Chronic inflammation, when unregulated, may be an underlying factor promoting the pathogenesis of a variety of diseases. Pro-inflammatory cytokines and chemokines, including IL-1, IL-6, IL-8, and TNF-α can establish an environment that promotes disease progression. These cytokines and chemoattractants are secreted by immune regulatory cells, but also by tumor cells, tumor-associated macrophages, and stromal cells. In addition, tumor-associated cells secrete growth factors, such as VEGF and FGF basic, and matrix-degrading proteases. Together these factors establish a microenvironment that promotes tumor progression by stimulating processes such as angiogenesis, invasion, and metastasis. Importantly, cytokines secreted by tumor cells also recruit tumor infiltrating leukocytes. These leukocytes can produce either pro- or anti-tumorigenic/angiogenic cytokines, which will also play a role in determining how tumor growth is affected.

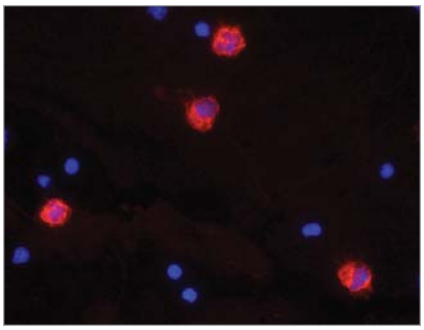
Other conditions associated with chronic inflammation, such as obesity, also lead to alterations in the levels of specific cytokines. Leptin, Hepatocyte Growth Factor (HGF), and Heparin-binding Epidermal Growth Factor-like Growth Factor (HB-EGF) are three cytokines secreted by adipose tissue or adipose-associated macrophages that are elevated in obese individuals. Overexpression of these cytokines has detrimental cellular effects that include stimulating proliferation and angiogenesis. In contrast, Adiponectin, which is reduced in obesity, negatively regulates these processes by inducing endothelial cell apoptosis. Changes in cytokine levels may help to explain the link between obesity and certain forms of cancer.



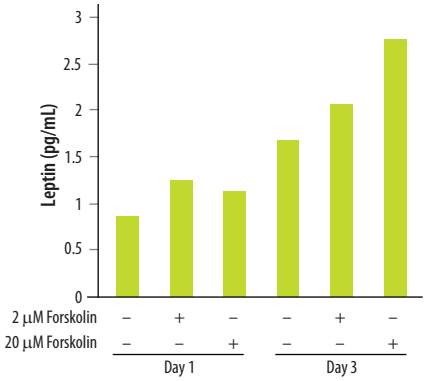
Cytokines, Chemokines, and Growth Factors Associated with the Tumor Microenvironment Stimulate Angiogenesis. Cytokines and chemokines released by inflammatory and tumor-associated cells provide a critical link between inflammation, angiogenesis, invasion, and metastasis. Tumor-associated macrophages (TAMs) accumulate in tumors, and along with stromal cells and tumor cells themselves, release angiogenic factors including VEGF, FGF basic, TNF-α, IL-1, IL-6, IL-8, CXCL12/SDF-1α, and proteases. These factors affect endothelial, epithelial, and mesenchymal cells in the tumor microenvironment and ultimately promote angiogenesis, invasion, and metastasis.



HB-EGF-induced Proliferation of Mouse Balb/3T3 Cells. Balb/3T3 mouse embryonic fibroblast cells were treated with increasing concentrations of recombinant human HB-EGF (Catalog # 259-HE) for four days. Cell proliferation was determined in a fluorometric assay using the redox sensitive dye, Resazurin (Catalog # AR002).



Detection of IL-1β in Human Peripheral Blood Mononuclear Cells (PBMCs). IL-1β was detected in LPS-stimulated human PBMCs using anti-human IL-1β polyclonal antibody (Catalog # AF-210-NA). The cells were stained using NorthernLights™ 557-conjugated secondary antibody (Catalog # NL001; red) and counterstained with DAPI (blue).



Assessment of Natural Leptin Levels in BeWo Cell Culture Supernatants using the Quantikine® ELISA Kit. Cell culture supernatants removed from BeWo human choriocarcinoma cells, one or three days following stimulation with 2 µM or 20 µM forskolin, were assayed using the Human Leptin Quantikine ELISA Kit (Catalog # DLP00).

PRODUCTS FOR CYTOKINE-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS	FLOW CYTOMETRY KITS	ELISPOT KITS & DEVELOPMENT MODULES
Adiponectin/Acrp30	H M	H M R	H M	H			
gAdiponectin/gAcrp30	H M						
Amphiregulin	H M	H M	H M				
EGF	H M R	H M R	H M R	H		H	
EGF R/ErbB1	H M	H M	H		H		
EMAP-II	M	H					
G-CSF	H M	H M	H M	H		H	
GM-CSF	H M R P Ca F	H M R P Ca F	H M R Ca F	H M R		H	H M F
HB-EGF	H	H	H				
HGF	H M Ca	H M Ca	H M	H			
HGF R/c-MET	H M Ca	H M Ca	H M Ca		H		
IFN-α	H M R P CR F Pr	H M P CR	H M				
IFN-α/β R1	M	H M R B Ca Pr					
IFN-α/β R2	H	H M					
IFN-β	H M R	H M R	H M				
IFN-γ	H M R P B Ca CR E F RM	H M R P B Ca CR E F RM	H M R P B Ca CR E F Pr	H M R			H M R P Ca E F Pr
IFN-γ R1/CD119	H M	H M	H M				
IFN-γ R2		H M					
IL-1α/IL-1F1	H M R P CR	H M R P CR	H M R	H R		H	
IL-1β/IL-1F2	H M R P Ca CR E F RM	H M R P Ca CR E F	H M R P F	H M R		H	H P
IL-1ra/IL-1F3	H M R P E	H M P E	H M	H			
IL-1 RI	H M R	H M	H				
IL-1 RII	H M	H M	H				

continued on next page.

PRODUCTS FOR CYTOKINE-RELATED RESEARCH *continued*

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS	FLOW CYTOMETRY KITS	ELISPOT KITS & DEVELOPMENT MODULES
IL-1 RAcP/IL-1 R3	H	H					
IL-4	H M R P B Ca CR E F RM	H M R P B Ca CR E F	H M R P CR E F	H M R		H	H M Ca E
IL-4 Rα	H M	H M					
IL-6	H M R P Ca CR E F	H M R P Ca CR E F	H M R P Ca F	H M R		H	H M R
IL-6 Rα	H M	H M	H M				
IL-10	H M R P Ca CR E F V	H M R P Ca CR E F V	H M R P Ca E F	H M R		H	H M Ca F
IL-10 Rα	H M	H M					
IL-10 Rβ	H	H M					
IL-12	H M R P Ca F RM	H M R P	H M	H M		M	
IL-12 p35		H P					
IL-12 p70		H M	H M	H M			
IL-12/IL-23 p40	H M Ca F	H M R P Ca F	H M P Ca F				
IL-12 Rβ1	H M	H M					
IL-12 Rβ2	H	H M					
IL-13	H M R RM	H M R	H M	H M R			H M
IL-13 Rα1	H M	H	H				
IL-13 Rα2	H M	H M	H				
IL-18/IL-1F4	H M R P RM	H M R P Ca RM	H M	R			
IL-18 Rα/IL-1 R5	H	H M					
IL-18 Rβ/IL-1 R7	H	H M					
IL-20 Rα	H	H M					
IL-20 Rβ	H M	H M					
IL-22 Rα1	H M	H M					
IL-24	H	H M	H				
Leptin/OB	H M R	H M	H M	H			
Leptin R	H M	H M	H M				
MIF	H M	H	H				
Oncostatin M/OSM	H M	H M	H				
PBEF		H M					
TGF-α	H	H	H				
TGF-β		Ms					
TGF-β1	H P	H Ms	H M R P Ca			H	H
TGF-β1, 2, 3		Ms		Ms			
TGF-β2	H P	Ms	H				
TGF-β3	H	Ms	H				
TGF-β RI/ALK-5	M	H M					
TGF-β RII	H M	H M					
TGF-β RIII	H M	H M					
TNF-α/TNFSF1A	H M R P B Ca CR E F Rb RM	H M R P B Ca CR E RM	H M R P B Ca E F Pr RM	H M R		H	H M P E Pr
TNF RI/TNFRSF1A	H M Ca	H M	H M				
TNF RII/TNFRSF1B	H M	H M	H M				

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

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

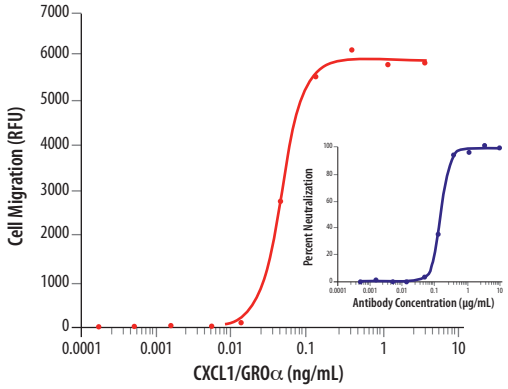
Chemokines & Receptors

Chemokines are a large superfamily of mostly small, secreted chemotactic cytokines that have a well-characterized ability to recruit leukocytes to sites of inflammation. A subset of these factors has also been found to be involved in regulating the angiogenic activities of endothelial cells under both physiological and pathological conditions such as wound healing, chronic inflammation, and tumor growth. Chemokines of the CXC family are characterized by a Cysteine-X-Cysteine (CXC) motif at their amino terminal ends and act as potent inducers or inhibitors of angiogenesis. Those containing the amino acids Glutamic acid-Leucine-Arginine (ELR) adjacent to the CXC motif bind to the CXCR2 receptor and promote angiogenesis, while those lacking the ELR motif primarily bind to CXCR3 and inhibit angiogenesis. A few of the CC chemokines, most notably CCL2, have also been shown to stimulate angiogenesis. Several studies demonstrate a correlation between the levels of angiogenic chemokines and tumor angiogenesis. Likewise, depletion of angiogenic chemokines in different forms of cancer has been shown to reduce tumor angiogenesis and metastatic potential.

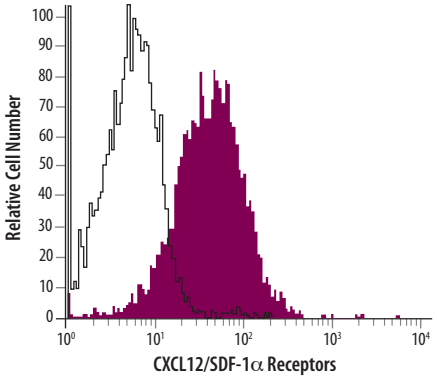
Angiogenic Chemokines	Receptors
CXCL1/GROα	CXCR2
CXCL2/GROβ	CXCR2
CXCL3/GROγ	CXCR2
CXCL5/ENA-78	CXCR2
CXCL6/GCP-2	CXCR2
CXCL7/NAP-2	CXCR2
CXCL8/IL-8	CXCR2
CCL2/MCP-1	CCR2
CCL11/Eotaxin	CCR3
CCL16/HCC-4	CCR1
CXCL12/SDF-1α	CXCR4

Angiostatic Chemokines	Receptors
CXCL4/PF4	CXCR3B
CXCL9/MIG	CXCR3B
CXCL10/IP-10	CXCR3B
CXCL11/I-TAC	CXCR3B

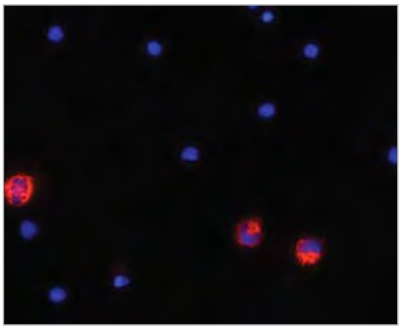
Angiogenic and Angiostatic Chemokines & Receptors. Specific CXC chemokines promote angiogenesis, while others have angiostatic effects. [Information in the table was adapted from Mehrad, B. *et al.* (2007) Thromb. Haemost. **97**:755. and Keeley, E. *et al.* (2008) Arterioscler. Thromb. Vasc. Biol. **28**:1928.]



CXCL1/GROα-induced Chemotaxis & Antibody Neutralization. BaF/3 mouse pro B cells transfected with human CXCR2 were placed in the upper compartments of a chemo-taxis chamber with increasing concentrations of recombinant human CXCL1/GROα (Catalog # 275-GR) placed in the lower compartments. Cell migration was monitored by staining the cells in the lower chamber with the redox sensitive dye, Resazurin (Catalog # AR002; orange line). The chemotactic effect induced by 10 ng/mL CXCL1/GROα was neutralized by pre-incubating the protein with increasing concentrations of anti-human CXCL1/GROα polyclonal antibody (Catalog # AF275) prior to its addition to the chemotaxis chamber (inset).

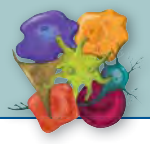


Detection of Cells Expressing CXCL12/SDF-1α Receptors by Flow Cytometry. The human CXCL12/SDF-1α Biotinylated Fluorokine® Kit (Catalog # NNS00) was used to stain CXCL12/SDF-1α receptor positive Jurkat human T cells. Cells were labeled with biotinylated recombinant human CXCL12/SDF-1α (filled histogram) or with an unrelated biotinylated protein (open histogram), and then stained with fluorescein-conjugated avidin.



Detection of CXCL8/IL-8 in Human Peripheral Blood Mononuclear Cells (PBMCs). Human PBMCs were activated with PMA, ionomycin, and monensin. CXCL8/IL-8 was detected using anti-human CXCL8/IL-8 monoclonal antibody (Catalog # MAB208). The cells were stained using NorthernLights™ 557-conjugated secondary antibody (Catalog # NL007; red) and counterstained with DAPI (blue).

PRODUCTS FOR CHEMOKINE-RELATED RESEARCH LISTED ON PAGE 30>>>



PRODUCTS FOR CHEMOKINE-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS	FLOW CYTOMETRY KITS	ELISPOT KITS & DEVELOPMENT MODULES
CCL2/JE/MCP-1	H M R Ca	H M Ca CR	H M Ca	H M		H M	
CCL5/RANTES	H M CR F	H M CR F	H M	H		H	H M
CCL11/Eotaxin	H M	H M	H M	H		H	
CCL16/HCC-4	H	H	H				
CCL23/Ckβ8-1	H	H	H				
CCR1		H					
CCR2		H					
CCR3		H M					
CCR5		H			H		
CX3CL1/Fractalkine	H M R	H M R	H M R			H	
CX3CR1		H M					
CXCL1/2/3/GRO	CR	H CR					
CXCL1/GROα/KC/CINC-1	H M R	H M R	H M R	M			
CXCL2/GROβ/MIP-2/CINC-3	H M R CR	H M R	M R	M R			
CXCL3/GROγ/CINC-2/DCIP-1	H M R	H R	R	R			
CXCL4/PF4	H M	H M	H M				
CXCL5/ENA-70	H						
CXCL5/ENA-74	H						
CXCL5/ENA-78	H	H	H	H			
CXCL6/GCP-2	H	H	H				
CXCL7/NAP-2	H	H	H				
CXCL8/IL-8	H P Ca F	H P Ca F	H P Ca F	H		H	H
CXCL9/MIG	H M	H M	H M				H
CXCL10/IP-10/CRG-2	H M CR	H M CR	H M	H			H
CXCL11/I-TAC	H M	H M	H M	H			
CXCL12/SDF-1	H M F RM	H M	H M			H	
CXCL14/BRAK	H M	H M	H				
CXCR1/IL-8 RA		H					
CXCR2/IL-8 RB		H M					
CXCR3		H M					
CXCR4		H M F			H		
CXCR7/RDC-1		H					
DARC		H					

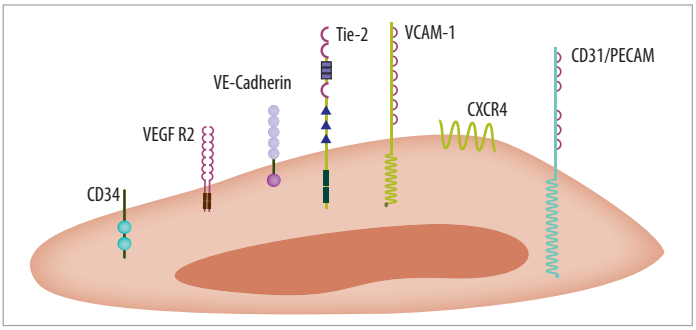
For more information on chemokine-related products, please visit our website at www.RnDSystems.com/go/Chemokine

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

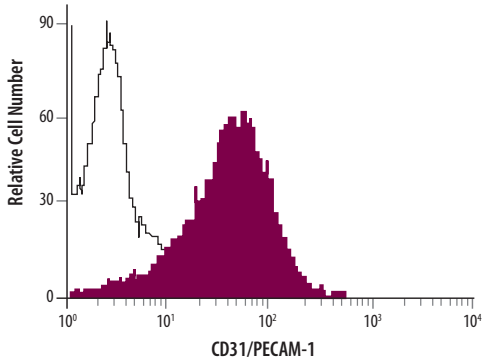
IX. Endothelial Cell Markers & Adhesion Molecules

Endothelial Cell Markers

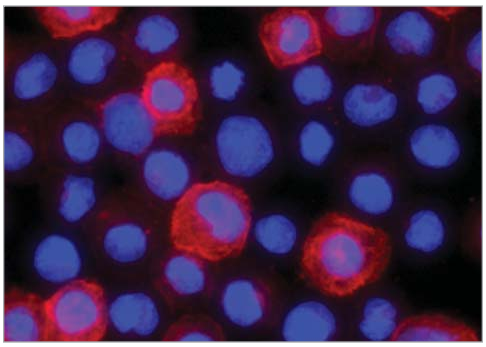
Endothelial cells line the interior of all blood vessels and are typically one of the most quiescent cell types in the body. These cells are derived from a common angioblast precursor and subsequently develop organ specific properties. Although embryonic endothelial cells exhibit much heterogeneity within and between organs, multiple proteins expressed by endothelial cells can be used to distinguish these cells from other cell types in the body. Differentiation of endothelial cells is governed by several factors, including the immediate microenvironment, interactions with surrounding cells, and the local release of cytokines and growth factors. Adult endothelial cells retain remarkable plasticity and are known to reprogram in response to IL-1, TNF, VEGF, and FGF. Endothelial cell dysfunction can promote the development of chronic inflammatory conditions and lead to vascular disease.



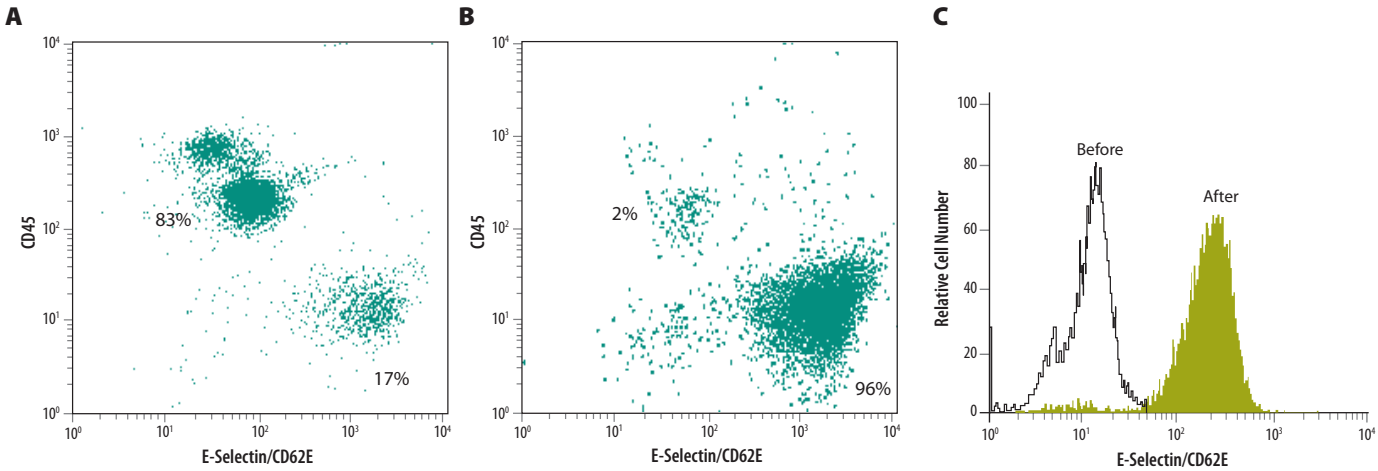
Endothelial Cells Express Cell Surface Markers that Distinguish them from Other Cell Types. Endothelial cells are the primary cells involved in mediating angiogenesis. Multiple proteins expressed on the surface of endothelial cells can be used to distinguish these from other cell types in the body.



Detection of CD31/PECAM-1 by Flow Cytometry. Human umbilical vein endothelial cells (HUVECs) were stained with APC-conjugated anti-human CD31/PECAM-1 monoclonal antibody (Catalog # FAB3567A; filled histogram) or with APC-conjugated mouse IgG_1 isotype control antibody (Catalog # IC002A; open histogram).



Cadherin-13 in Human Lung Cancer Cells. Cadherin-13 was detected in the NCI-H460 human lung cancer cell line using anti-human Cadherin-13 polyclonal antibody (Catalog # AF3264). Cells were stained using NorthernLights-557-conjugated IgG secondary antibody (Catalog # NL001; red) and counterstained with DAPI (blue).

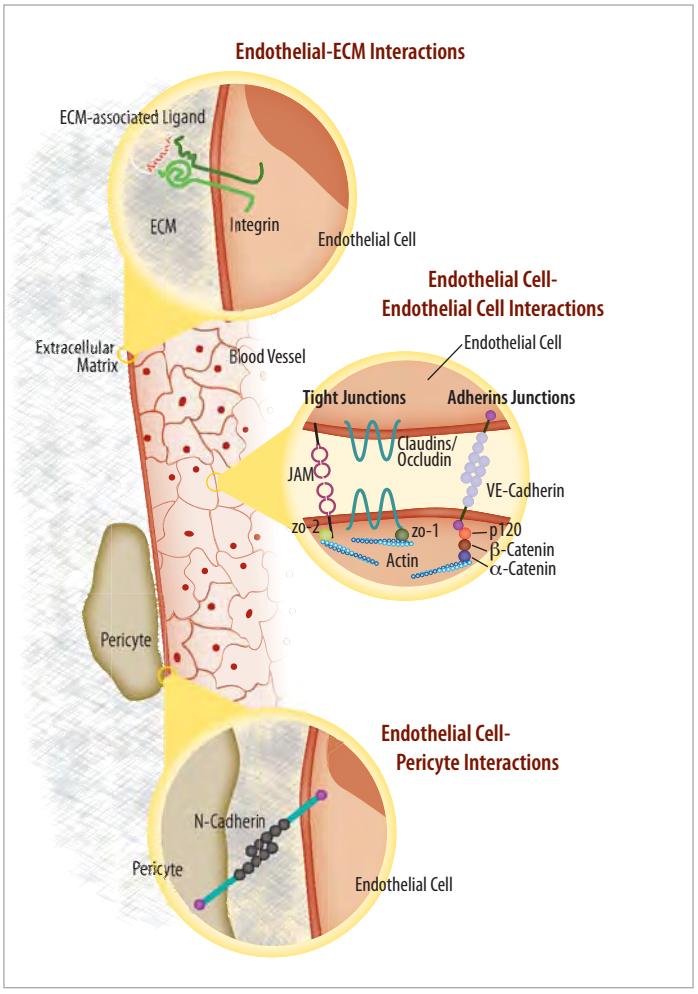


Isolation of E-Selectin/CD62E⁺ Cells Using the PlusCellect™ Kit. E-Selectin/CD62E⁺ cells were isolated from a culture containing peripheral blood mononuclear cells (PBMCs) spiked with TNF-α-activated human vein endothelial cells (HUVECs) by positive selection using the Human E-Selectin/CD62 PlusCellect Kit (Catalog # PLS724). Cells were stained with CFS-conjugated anti-human E-Selectin monoclonal antibody (provided in the kit) before **(A)** and after **(B)** selection and with APC-conjugated anti-human CD45 monoclonal antibody (Catalog # FAB1430A) to detect contaminating blood cells. A histogram depicting data from the E-Selectin/CD62E⁺ staining in **(A)** and **(B)** is also shown **(C)**.

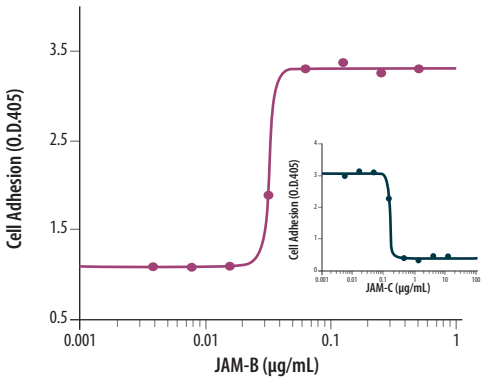
Adhesion Molecules & Angiogenesis

Substantial changes in the adhesive interactions between cells and the extracellular matrix (ECM) take place to permit endothelial cell migration during angiogenesis. These interactions are mediated by Integrins. Integrins are transmembrane, heterodimeric receptors that bind to ligands in the ECM to either promote endothelial cell motility and proliferation, or to inhibit endothelial cell activities. Interactions between specific Integrins and growth factor receptors such as FGF R, VEGF R2, and PDGF R can enhance FGF-, VEGF-, and PDGF-mediated pro-angiogenic effects on endothelial cells. Many of the Integrins that mediate physiological angiogenesis are also involved in regulating angiogenesis under pathological conditions including Integrins $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha V\beta 3$, and $\alpha V\beta 5$. Inhibition of either Integrin $\alpha V\beta 3$ or Integrin $\alpha V\beta 5$ has been shown to prevent angiogenesis and tumor growth in animal models.

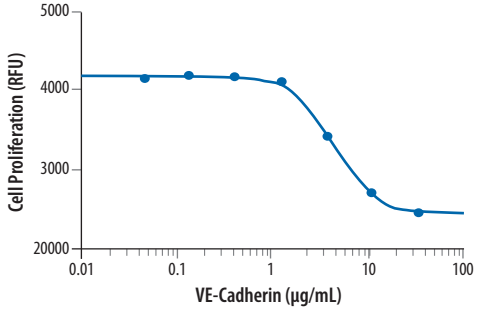
Endothelial cell-cell interactions are important for the maintenance of vascular integrity. Adherens and tight junctions seal adjacent endothelial cells together through specific protein-protein interactions. Tight junctions are mediated by Claudins, Occludin, JAM family proteins, and ESAM (endothelial cell specific adhesion molecule), while adherens junctions are primarily regulated by VE-Cadherin. Homophilic interactions between VE-Cadherin molecules on adjacent endothelial cells promote contact inhibition of endothelial cell growth, inhibit endothelial cell apoptosis, maintain the endothelial cell barrier, and protect the integrity of vessel walls. These adhesive functions require the intracellular association of Cadherins with Catenins, which are linked to the actin cytoskeleton. Abnormal increases in vascular permeability are associated with pathological angiogenesis. This can be caused by the loss of VE-Cadherin at the cell surface, phosphorylation-induced disruption of VE-Cadherin/Catenin complexes, crosstalk with tight junctions, or mechanical stress. The importance of VE-Cadherin in angiogenesis is highlighted by the fact that disruption of the gene results in embryonic lethality in mice accompanied by an inability of endothelial cells to properly organize into vessels. Another member of the Cadherin family, N-Cadherin, regulates interactions between endothelial cells and pericytes.



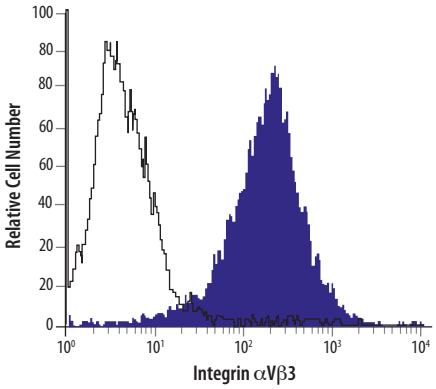
Adhesion Molecules Regulate Endothelial Cell Interactions with the Extracellular Matrix, Pericytes, and Adjacent Endothelial Cells. Endothelial cell interactions are important for the maintenance of vascular integrity. Changes in these interactions occur during angiogenesis to permit endothelial cell migration. Integrins mediate interactions between endothelial cells and the extracellular matrix. During angiogenesis, Integrins also form a complex with VEGF R2 that enhances the biological response of endothelial cells to VEGF. Integrins can also bind to other pro- and anti-angiogenic modulators to regulate angiogenesis. VE-Cadherin is the main adhesive protein in adherens junctions that mediates endothelial cell-cell interactions and vascular permeability. Cadherins associate with intracellular Catenin proteins to mediate their adhesive functions. Like Integrins, VE-Cadherin can also associate with growth factor receptors, but these associations typically inhibit proliferation rather than enhance it. N-Cadherin regulates endothelial cell-pericyte adhesion.



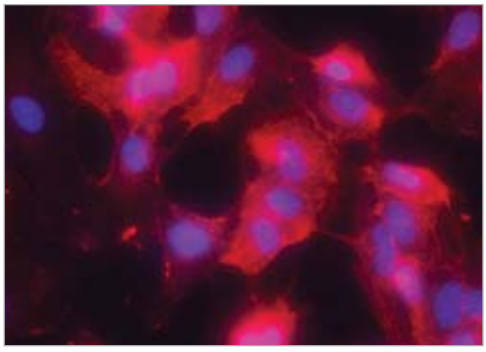
JAM-B-induced Adhesion of J45.01 Cells & Inhibition by JAM-C. J45.01 human leukemic T cells were incubated in microplate wells coated with the indicated concentrations of recombinant human JAM-B (Catalog # 1074-VJ). After two hours, non-adherent cells were washed off and attached cells were detected by measuring acid phosphatase activity (purple line). The adhesive effects mediated by 0.2 µg/mL recombinant human JAM-B were inhibited by the addition of increasing concentrations of recombinant human JAM-C (Catalog # 1189-J3; inset).



VE-Cadherin-mediated Inhibition of LL/2 Cell Proliferation. LL/2 mouse Lewis lung carcinoma cells were incubated with increasing concentrations of recombinant mouse VE-Cadherin (Catalog # 1002-VC). Relative cell proliferation was determined in a fluorometric assay using the redox sensitive dye, Resazurin (Catalog # AR002).

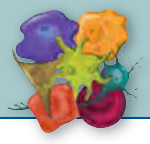


Detection of Integrin αVβ3 by Flow Cytometry. Human umbilical vein endothelial cells (HUVECs) were stained with PE-conjugated anti-human Integrin αVβ3 monoclonal antibody (Catalog # FAB3050P; filled histogram) or with PE-conjugated mouse IgG_1 isotype control antibody (Catalog # IC002P; open histogram).



ESAM Expression in Human Umbilical Vein Endothelial Cells (HUVECs). Endothelial cell adhesion molecule was detected in HUVECs using anti-human ESAM monoclonal antibody (Catalog # MAB4204). Cells were stained using NorthernLights™ 557-conjugated IgG secondary antibody (Catalog # NL007; red) and counterstained with DAPI (blue).

PRODUCTS FOR ENDOTHELIAL CELL MARKERS & CELL ADHESION RESEARCH LISTED ON PAGE 34>>>



PRODUCTS FOR ENDOTHELIAL CELL MARKERS & CELL ADHESION RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS
C1q R1/CD93		H M			
Cadherin-4/R-Cadherin	H	H			
Cadherin-13		H			
E-Cadherin	H M	H M	H M		H M
N-Cadherin	H	H			
VE-Cadherin	H M	H M	H		H
CD9		H M			
CD31/PECAM-1	H M P	H M P			H
CD34		R P Ca			
CD36/SR-B3	H M	H M	M		
CD47		H M			
CD151		H M			
CD160		M			
CEACAM-1/CD66a	H	H	H		
Claudin-3		H			
Coagulation Factor III/Tissue Factor	H M	H M	H		
D6		H			
DC-SIGNR/CD299	H	H			
EDG-1		H			
EDG-5		H			
EDG-8		H			
Endoglin/CD105	H M P	H M	H M		H
Endomucin		M			
EPCR		H M	H		
Erythropoietin R	H M	H M	H		
ESAM	H	H M			
Galectin-1	H M	H M	M		
Galectin-3	H M	H M	H M		
Galectin-3BP/MAC-2BP	H M	H			
ICAM-1/CD54	H M R	H M R	H M R	H R	
ICAM-2/CD102	H M	H M			
ILK		H M R			
Integrin α1/CD49a		H			
Integrin α2/CD49b		H M			
Integrin α2β1	H				
Integrin α4/CD49d		H M			
Integrin α4β1	H				
Integrin α4β7/LPAM-1	H				
Integrin α5/CD49e		H M			
Integrin α5β1	H				
Integrin α6β4	H				
Integrin α9		M			

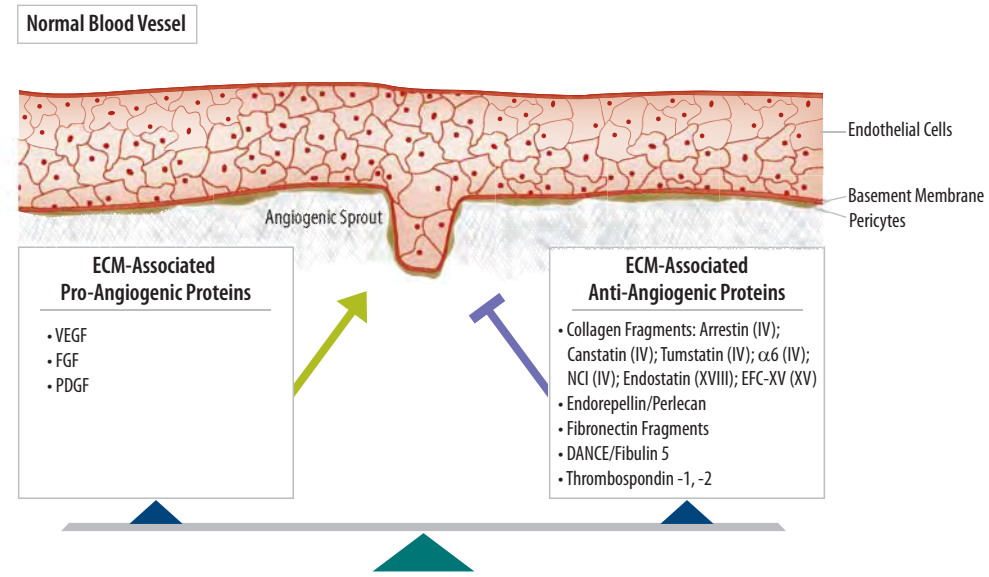
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS
Integrin α9β1	H				
Integrin αV/CD51		H			H
Integrin αVβ3	H	H			
Integrin αVβ5		H			
Integrin β1/CD29		H M			
Integrin β2/CD18	H	H M			
Integrin β3/CD61		H			
Integrin β4/CD104		H M			
Integrin β5		H			
JAM-A	H M	H M	M		
JAM-B/VE-JAM	H M	H M			
JAM-C	H M	H M			
LOX-1/SR-E1	H M	H M	H M		
MCAM/CD146		H R			H
MFG-E8	H M	H M	M		
Nectin-2/CD112	H M	H			
Nepmucin/CD300LG		M			
NrCAM	H	H	H		
Osteopontin/OPN	H M B	H M	H M		
Periostin/OSF-2	H M	H M	M		
Podocalyxin		H M			H
Podoplanin	H	H M			
E-Selectin/CD62E	H M R	H M R	H M	H	H
P-Selectin/CD62P	H M	H M	H M	H	
Stabilin-1		H			
Stabilin-2	H	H			
VE-Statin		H M			
TEM7		H			
TEM8		H			
Thrombomodulin/CD141	H M	H M	H		
THSD1	M	H M			
VAP-1/AOC3		H			
VCAM-1/CD106	H M	H M	H M	H	H
VG5Q	H	M			
vWF-A2	H	H			

For more information on products related to Endothelial Cell Markers, please visit our website at www.RnDSystems.com/go/ECMarkers

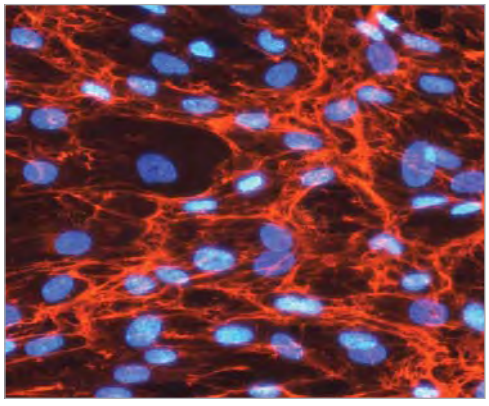
For more information on products related to Adhesion Proteins, please visit our website at www.RnDSystems.com/go/CellAdhesion

X. Extracellular Matrix-related Molecules

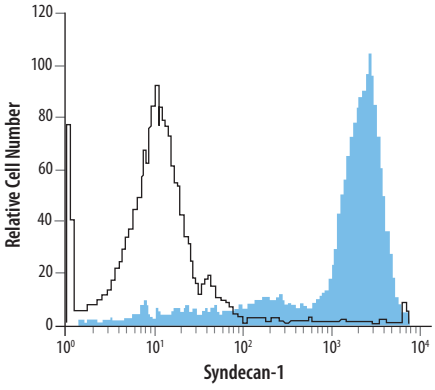
The extracellular matrix (ECM) plays an important role in angiogenesis by providing the structural support necessary for blood vessel formation and supplying multiple endogenous pro- and anti-angiogenic factors that regulate endothelial cell survival and vessel stability. Pro- and anti-angiogenic factors in the ECM can directly interact with Integrins on the surface of vascular endothelial cells to regulate endothelial cell activities. During angiogenesis, the ECM basement membrane is degraded by proteases that promote endothelial cell migration. These proteases also stimulate the release of pro-angiogenic factors from the ECM including VEGF and FGF basic. In contrast, proteins or protein fragments that inhibit endothelial cell proliferation, migration, and tube formation are also associated with the ECM. These endogenous inhibitors of angiogenesis include proteins such as Perlecan, Endostatin, DANCE/Fibulin 5, Thrombospondins, and fragments of Collagen and Fibronectin. The levels of pro- and anti-angiogenic factors in the ECM are critical for determining whether new vessels can be stably formed.



Multiple Pro- and Anti-Angiogenic Factors Are Associated with the Extracellular Matrix. The extracellular matrix (ECM) provides structural support for blood vessels and is also a source of multiple pro- and anti-angiogenic proteins that can modulate endothelial cell activity. Typically in adult humans, there is a balance between pro- and anti-angiogenic factors that keeps angiogenesis switched "off". Disruption of this balance occurs during physiological processes such as wound healing and pregnancy, as well as during the progression of pathological conditions such as tumor growth. Altering the balance between pro- and anti-angiogenic factors can turn the angiogenic switch "on". [Figure adapted from Nyberg, P. *et al.* (2005) Cancer Res. **65**:3967.]



Fibronectin Expression in WS1 Cells. Fibronectin was detected in WS1 human fibroblast cells using anti-human Fibronectin monoclonal antibody (Catalog # MAB19181) followed by NorthernLights™ 557-conjugated anti-mouse IgG (Catalog # NL007; red). Cells were counterstained with DAPI (blue).



Detection of Syndecan-1 by Flow Cytometry. RPMI 8226 human myeloma cells were stained with APC-conjugated anti-human Syndecan-1 monoclonal antibody (Catalog # FAB2780A; filled histogram) or with APC-conjugated rat IgG_1 isotype control antibody (Catalog # IC005A; open histogram).

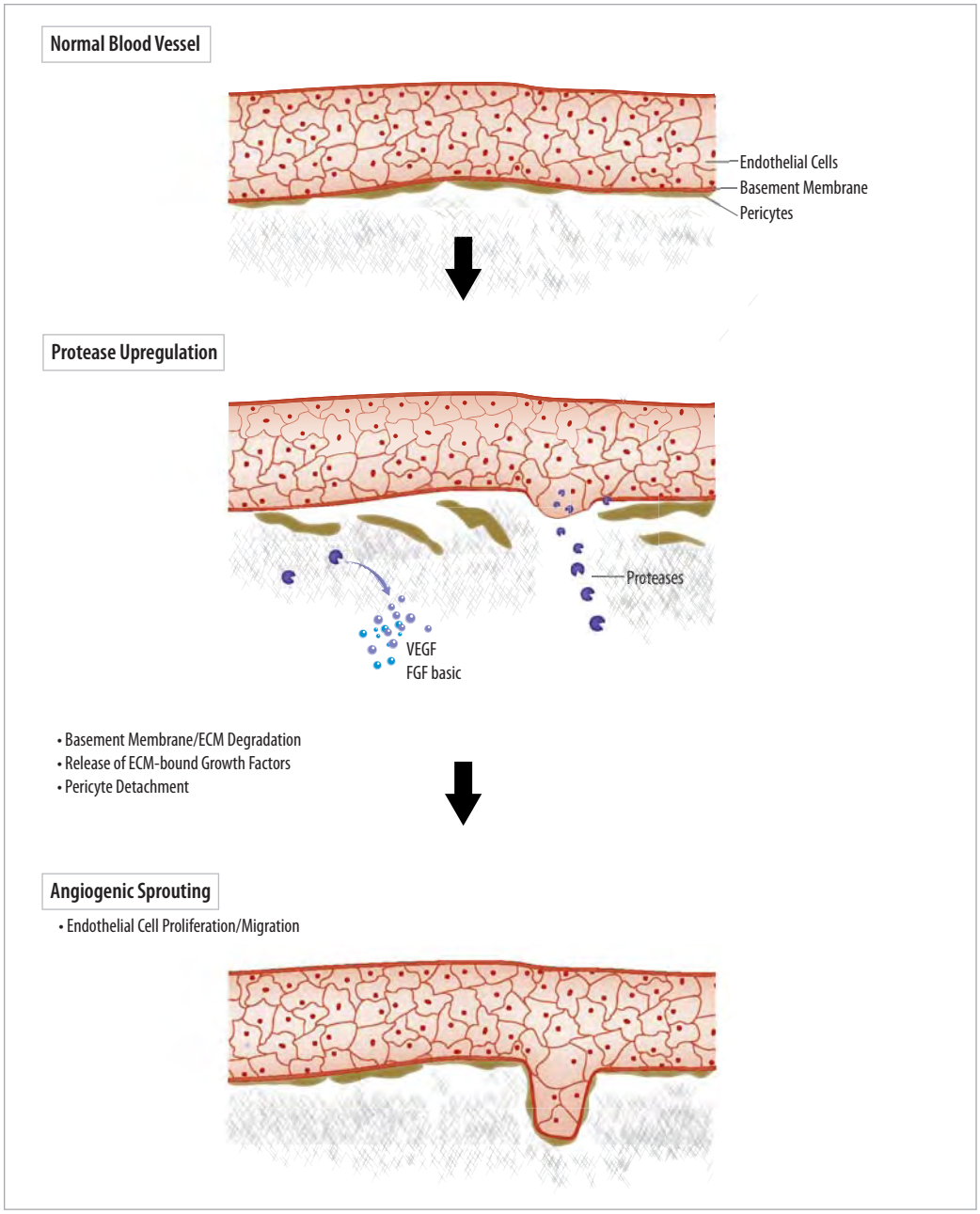
PRODUCTS FOR EXTRACELLULAR MATRIX RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	CELL SELECTION & DETECTION KITS & REAGENTS	CELL CULTURE PRODUCTS
C4.4A/LYPD3	H M	H M				
CD44	H	H Ca			H	
Collagen I						R B Ms
Collagen IV						M Ms
CTGF/CCN2		H				
Cyr61/CCN1	H	H M				
DANCE		H				
Decorin	H M	H M	H M			
ECM-1	H	H M				
Endocan/ESM-1	H M	H M	M			
Endorepellin/Perlecan		H				
Endostatin		H M	H	H		
Fibronectin	H B	H				H B
F-Spondin	H	H				
Glypican 1	H M	H				
Glypican 3	H	H				
Hyaluronan	Ms		Ms			
Laminin α1		H M				
Laminin α4		M				
Laminin γ1		H R				
Laminin S		H R Ch				
Laminin-1		M				
Laminin-5		H				
Layilin	H M	H M	H			
Lumican		H M				
Mindin		H				
NG2/MCSP		H				
NOV/CCN3	H M	H M	H M			
SMOC-1		M R				
SMOC-2		H M				
SPARC/Osteonectin	H M	H M				
SPARC-like 1/SPARCL1	H M	H M				
Syndecan-1/CD138		H M				
Syndecan-2	H	H				
Syndecan-3		H M				
Syndecan-4	H	H				
Tenascin C	H	H M				
Thrombospondin-1	H	H	H			
Thrombospondin-2	H	H	H	H		
Thrombospondin-4	H	H				
Versican		H				
Vitronectin	H B	H M				H B
WISP-1/CCN4	H M	H M				

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

XI. Proteases & Associated Molecules

Angiogenesis is associated with upregulation of proteases that degrade components of the extracellular matrix (ECM) to permit endothelial cell migration and new vessel formation. These proteases also modify cell adhesion, induce the release of angiogenic factors from the ECM, and are involved in processing growth factors and cytokines. Several families of proteases produced by stromal cells, activated endothelial cells, tumor cells, or tumor-associated macrophages have been implicated in these processes including matrix metalloproteinases (MMPs), aminopeptidases, serine proteases such as uPA, Plasmin, and Plasma Kallikrein, and the cathepsin cysteine proteases. Inhibitors of these proteases are also important as the ratio between the pro-angiogenic proteases and their inhibitors may determine whether new vessel formation can occur.

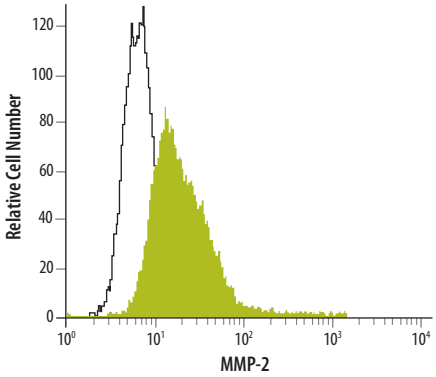


Protease Activity Is Required For Angiogenesis. Angiogenesis is an invasive process that requires proteolytic degradation of the basement membrane and extracellular matrix (ECM) to allow endothelial cell migration and proliferation in the surrounding tissue. Multiple families of proteases have been implicated in these processes. In addition, proteases induced during angiogenesis promote the mobilization of growth factors such as VEGF and FGF basic from the ECM, and are involved in processing matrix proteins and cytokines that activate or inhibit angiogenesis. Although quiescent endothelial cells have little protease activity, protease upregulation is associated with wound healing, inflammation, and tumor growth.

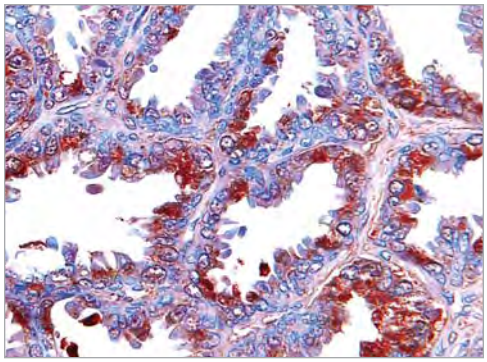
MMPs & TIMPs

Matrix Metalloproteinases (MMPs) are members of a family of Zn^{2+}-dependent endopeptidases that play important roles in multiple physiological and pathological processes. Most MMPs are secreted, with the exception of membrane-type MMPs (MT-MMPs), which are anchored to the cell membrane. MMPs are synthesized as latent proenzymes and are activated by proteolytic cleavage, typically following secretion. Upon activation, MMPs catalyze the breakdown of the extracellular matrix (ECM) by cleaving multiple ECM proteins including different types of Collagen, Laminin, Fibronectin, Aggrecan, and Perlecan. Degradation of the ECM allows endothelial cells to invade the surrounding tissue. MMP-2, -9, and -14 (MT1-MMP) have all been implicated in the regulation of angiogenesis. In addition to degrading components of the ECM, MMPs are involved in processing cytokines and promoting the release of VEGF and FGF basic from the ECM. Significantly, MMPs can act as negative regulators of angiogenesis as well by cleaving Plasminogen and Collagen XVIII to produce Angiostatin and Endostatin, two well-characterized angiogenic inhibitors.

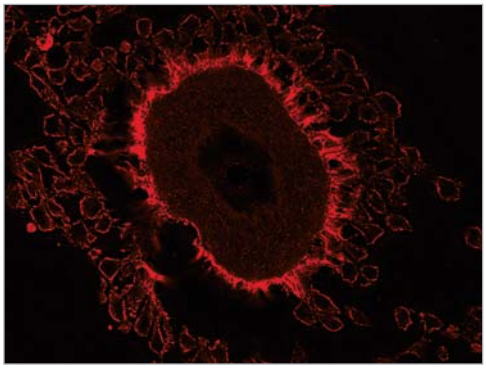
MMP activities are regulated on several levels including transcription, pro-enzyme activation, or by their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). The TIMP family consists of four family members, TIMP-1-4, which mediate the balance between ECM degradation and deposition. Increased MMP activity and accelerated ECM degradation are associated with pathological conditions such as cancer.



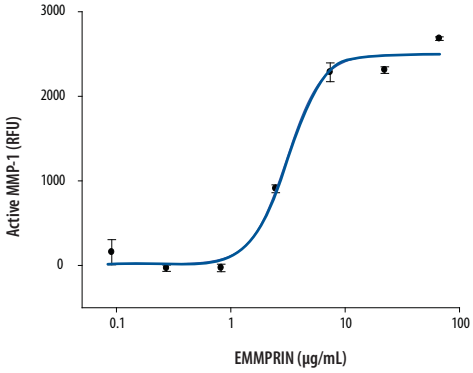
Intracellular Detection of MMP-2 by Flow Cytometry. Intracellular MMP-2 was detected in MG-63 human osteosarcoma cells with PE-conjugated anti-human MMP-2 monoclonal antibody (Catalog # IC9023P; filled histogram) or with PE-conjugated mouse IgG_{2A} isotype control antibody (Catalog # IC003P; open histogram).



MMP-14 in Human Benign Nodular Hyperplasia. MMP-14 was detected in a paraffin-embedded tissue section of human benign nodular hyperplasia using mouse anti-human MMP-14 monoclonal antibody (Catalog # MAB918) followed by avidin-biotin amplification. Labeling with the MMP-14 antibody (red) is observed in epithelial cells lining the glandular lumen. Tissues were counterstained with hematoxylin (blue).

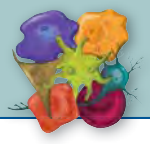


TIMP-1 in Mouse Ovary. TIMP-1 was detected in a frozen section of mouse ovary using anti-mouse TIMP-1 polyclonal antibody (Catalog # AF980; red).



EMMPRIN-stimulated MMP-1 Production. Normal human lung fibroblast cells were cultured on microplates coated with increasing concentrations of recombinant human EMMPRIN/Fc chimera (Catalog # 972-EMN) for 3 days. EMMPRIN stimulated a dose-dependent increase in the levels of active MMP-1 in cell culture supernatants as measured using the human Active MMP-1 Fluorokine® E Kit (Catalog # F1M00).

PRODUCTS FOR MMP-RELATED RESEARCH LISTED ON PAGE 40>>>



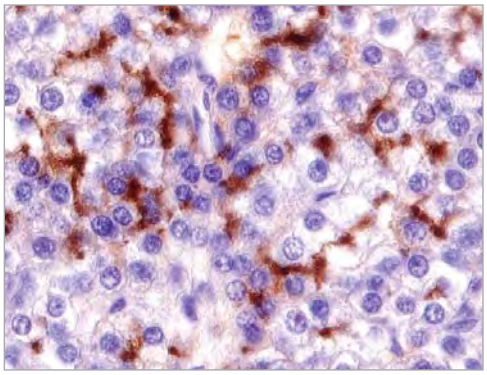
PRODUCTS FOR MMP-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS	ELISPOT KITS & DEVELOPMENT MODULES	PROTEASE ACTIVITY ASSAYS & REAGENTS
α2-Macroglobulin	H	H				
ECM-1	H	H M				
EMMPRIN	H	H M	H			
Lipocalin-2/NGAL	H M R	H M R	H M			
MMP-1	H	H	H	H	H	H
MMP-2	H M R	H M R	H	H		
MMP-3	H M	H M	H M	H	H	
MMP-7	H M	H M	H	H	H	
MMP-8	H M R	H R	H	H		
MMP-9	H M R	H M	H M	H	H M	H
MMP-10	H	H	H			
MMP-11		H				
MMP-12	H M	H M		H		
MMP-13	H	H	H	H		H
MMP-14	H	H				
MMP-15		H				
MMP-16/MT3-MMP	H	H				
MMP-24/MT5-MMP	H M	H M				
MMP-25/MT6-MMP		H				
MMP-26		H				
RECK		H				
Testican 1/SPOCK1	H	H				
Testican 2/SPOCK2	H	H				
Testican 3/SPOCK3	M	M	M			
TIMP				H		
TIMP-1	H M R	H M R	H M R	R	H	
TIMP-2	H	H	H			
TIMP-3	H	H	H			
TIMP-4	H	H	H			
TRA-1-85		H				

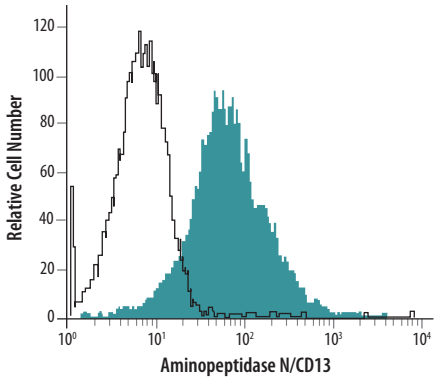
For more information on MMP-related products, please visit our website at www.RnDSystems.com/go/MMP

Aminopeptidases

Aminopeptidases are exopeptidases that modify the N-terminus of proteins and peptides to regulate their activation or degradation. Many aminopeptidases are members of two metalloprotease families (M01 and M24). They have distinct substrate specificities, cellular location(s), and inhibitor sensitivities. Several studies suggest that these enzymes are involved in tumor angiogenesis. Aminopeptidase N has been found to be overexpressed in tumor cells, while inhibition of Methionine Aminopeptidase has been shown to inhibit angiogenesis and tumor growth.



Aminopeptidase N/CD13 Expression in Human Liver Cancer. Aminopeptidase N/CD13 was detected in paraffin-embedded human liver cancer sections using anti-human Aminopeptidase N monoclonal antibody (Catalog # MAB3815). Tissues were stained using the anti-mouse HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS002; brown) and counterstained with hematoxylin (blue).



Detection of Aminopeptidase N/CD13 by Flow Cytometry. bEND.3 mouse brain-derived endothelial cells were stained with PE-conjugated anti-mouse Aminopeptidase N/CD13 polyclonal antibody (Catalog # FAB2235P; filled histogram) or with PE-conjugated normal goat IgG (Catalog # IC108P; open histogram).

PRODUCTS FOR AMINOPEPTIDASE-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES
Aminopeptidase A/ENPEP	H	M
Aminopeptidase LRAP/ERAP2	H	H
Aminopeptidase N/ANPEP	H M	H M
Aminopeptidase O/ONPEP		H
Aminopeptidase P1/XPNPEP1	H	H
Aminopeptidase P2/XPNPEP2*	H M	H M
Aminopeptidase PILS/ARTS1	H M	H M
Leukotriene A4 Hydrolase/LTA4H	H	
Methionine Aminopeptidase	*E. coli*	
Methionine Aminopeptidase 1	H	H
Methionine Aminopeptidase 1D/MAP1D	H	H
Methionine Aminopeptidase 2	H	H
PA2G4		H
TRH-degrading Ectoenzyme/TRHDE	M	M R

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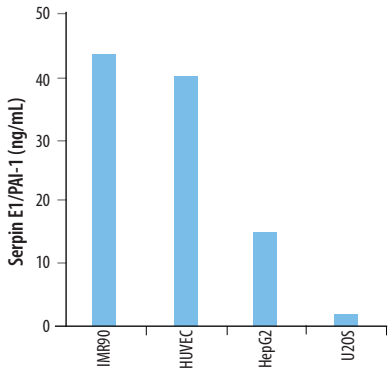
For more information on Aminopeptidase-related products, please visit our website at www.RnDSystems.com/go/Aminopeptidases

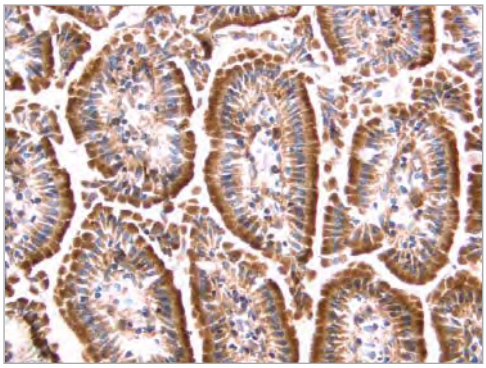
Plasmin/Plasminogen & Kallikrein/Kinin Systems

Plasmin is a serine protease that is physiologically required for fibrinolysis to prevent excessive blood clotting. In addition, it has been found to be involved in promoting angiogenesis and tumor metastasis by stimulating basement membrane degradation, ECM remodeling, and activation of growth factors (including latent TGF-β and VEGF) and metalloproteinases. In the presence of fibrin, plasmin is formed by cleavage of plasminogen by one of two serine proteases, urokinase plasminogen activator (uPA) or tissue-type plasminogen activator (tPA), which bind to cell surface receptors, uPAR or Annexin II respectively. The uPA-uPAR system can also affect cell adhesion and motility and has itself been implicated in tumor cell invasion, angiogenesis, and metastasis. Serpin E1/PAI-1 and Serpin F2/α_2-antiplasmin are endogenous inhibitors of components of the plasmin activation system. Serpin E1/PAI-1 regulates plasmin formation by inhibiting both uPA and tPA, while Serpin F2/α_2-antiplasmin inhibits plasmin directly.

Components of the Kallikrein/Kinin system may also be involved in regulating pathological angiogenesis. The cleavage of high molecular weight (HMW) kininogen by the serine protease, plasma kallikrein, on endothelial cells generates bradykinin and a derivative of HMW kininogen, HKa. Bradykinin upregulates FGF basic and VEGF and promotes angiogenesis, while HKa induces endothelial cell apoptosis to inhibit angiogenesis. Aberrant expression of tissue kallikreins in different cancer types suggests that these proteases may also play an important role in disease progression. Tissue kallikreins may directly or indirectly promote angiogenesis by mediating ECM degradation through MMP or uPA/uPAR activation.



Assessment of Serpin E1/PAI-1 Levels in Cell Culture Supernatants using the Quantikine® ELISA Kit. Cell culture supernatants removed from IMR-90 primary human lung fibroblast cells, human umbilical vein endothelial cells (HUVEC), HepG2 human hepatocellular liver carcinoma cells, and U2OS human osteosarcoma cells were assayed using the Human Serpin E1/PAI-1 Quantikine ELISA Kit (Catalog # DSE100).



Serpin F2 Expression in Mouse Intestine. Serpin F2 was detected in cryostat tissue sections of mouse intestine using anti-mouse Serpin F2 polyclonal antibody (Catalog # AF1239). Tissues were stained using the anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

PRODUCTS FOR PLASMIN- & KALLIKREIN-RELATED RESEARCH

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
Angiostatin		H		
Annexin A2		H M R		
Kallikrein 1	H	H		
Kallikrein 3/PSA	H	H	H	
Kallikrein 4	H	H		
Kallikrein 5	H	H		
Kallikrein 13	H	H		
Kallikrein 14	H	H	H	
Plasma Kallikrein/KLKB1	H M	M		
Kininogen	H M	H M		
Kininogen/Kininostatin		H		

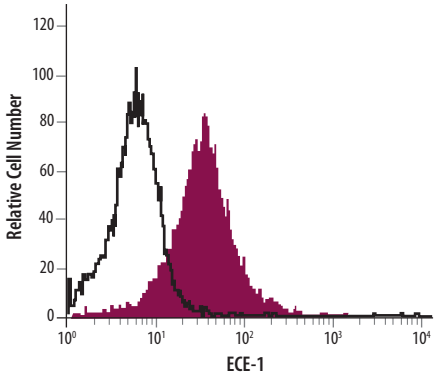
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
Kininostatin	H	H		
Phosphoglycerate kinase-1/PGK-1	H			
Plasminogen	H	H		
S100A10		H M		
Serpin A4/Kallistatin	H	H	H	
Serpin E1/PAI-1	H	H M	H	H
Serpin F2	H M	H M		
u-Plasminogen Activator (uPA)/Urokinase	H	H	H	
uPAR	H M	H M	H	

For more information on products related to the Plasmin/Plasminogen & Kallikrein/Kinin Systems, please visit our website at www.RnDSystems.com/go/Plasmin

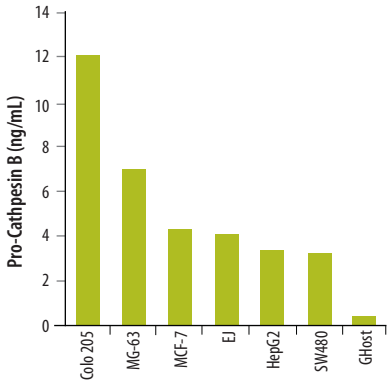
Other Proteases & Associated Molecules Involved in Angiogenesis

Several other proteases are also likely involved in angiogenesis-related ECM degradation and remodeling, including Cathepsins and metalloproteases belonging to the ADAM and ADAMTS families. Cathepsins are lysosomal proteases that are synthesized as inactive proenzymes. Like MMPs, they undergo processing to generate a mature, active form. Most human Cathepsins are cysteine proteases including Cathepsin B, C, F, H, K, L, O, S, V, W, X, while Cathepsin A and G are serine proteases, and Cathepsin D and E are aspartic proteases. Elevated levels of some of these proteases (Cathepsin B, H, and L) have been found in a variety of cancers, along with downregulation of their endogenous inhibitors, the Cystatins.

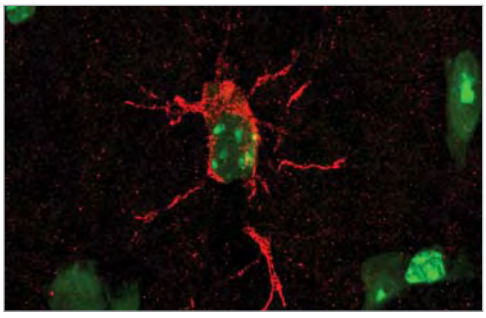
The levels of other proteases including members of the membrane-tethered ADAM (A Disintegrin and Metalloprotease) and secreted ADAMTS (A Disintegrin and Metalloprotease with Thrombospondin Motifs) families have also been found to be altered in a number of different tumor types. ADAM and ADAMTS metalloproteases regulate the activities of cytokines, growth factors, and Integrins to influence cellular functions such as adhesion, migration, and proliferation. They can have both pro- and anti-angiogenic effects. In addition, members of the Neprilysin family of zinc metalloproteases (ECE-1, ECE-2, Kell, Neprilysin, and others) have also been found to affect angiogenesis by activating or inhibiting proteins such as Endothelin-1 and FGF-2.



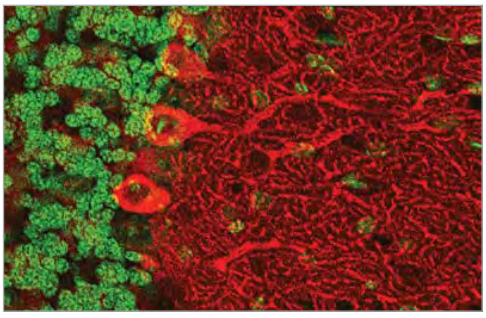
Intracellular Detection of ECE-1 by Flow Cytometry. MCF-7 breast adenocarcinoma cells were stained with anti-human ECE-1 monoclonal antibody (Catalog # MAB17841; filled histogram) or with rat IgG_1 isotype control antibody (Catalog # MAB005; open histogram) followed by APC-conjugated anti-rat IgG (Catalog # F0113).



Assessment of Pro-Cathepsin B Levels in Cancer Cell Lysates using the Quantikine® ELISA Kit. Cell lysates prepared from a series of different human cancer cell types including Colo 205 colon adenocarcinoma, MG-63 osteosarcoma, MCF-7 breast adenocarcinoma, EJ bladder carcinoma, HepG2 hepatocellular liver carcinoma, SW480 colon adenocarcinoma, and GHost cell carcinoma were assayed using the Human Pro-Cathepsin B Quantikine ELISA Kit (Catalog # DCATB0).

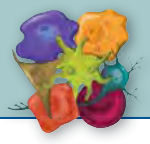


Detection of Neprilysin in Mouse Brain. Neprilysin was detected in cryostat tissue sections of mouse brain using anti-mouse Neprilysin polyclonal antibody (Catalog # AF1126; red). Tissues were counterstained (green).



ADAM15 Expression in Mouse Cerebellum. ADAM15 was detected in cryostat sections of mouse cerebellar Purkinje cells using anti-mouse ADAM15 polyclonal antibody (Catalog # AF945; red) and counterstained (green).

PRODUCTS FOR OTHER PROTEASE MOLECULES RELATED TO ANGIOGENESIS LISTED ON PAGE 44>>>



PRODUCTS FOR OTHER PROTEASE MOLECULES RELATED TO ANGIOGENESIS

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS
ACE/CD143	H M	H M	H M
ADAM8	H	H	H
ADAM9	H M	H M	H
ADAM12	H	H	
ADAM15	H	H M	
TACE/ADAM17	H M	H	H
ADAM19		H M	
ADAMTS1	H	H	
ADAMTS4	H	H	
ADAMTS5	H	H	
Carboxypeptidase B1	H M	H M	
Cathepsin B	H M	H M	H
Cathepsin D	H M	H M	
Cathepsin L	H M	H M I	H
Cathepsin S	H	H	H
Cathepsin X/Z/P	H M	H M	

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS
Cystatin A	H	H	
Cystatin B	H M	H M	
Cystatin C	H M	H M	H
Cystatin D	H	H	
Cystatin E/M	H M	H M	
Cystatin F	H M	H	
Cystatin H		M	
Cystatin H2		M	
Cystatin S	H	H	
Cystatin SA	H	H	
Cystatin SN	H	H	
DPPIV/CD26	H M	H M	H M
ECE-1	H M	H	
ECE-2	H	H	
Kell	M	H M	
Neprilysin/CD10	H M	H M	H M

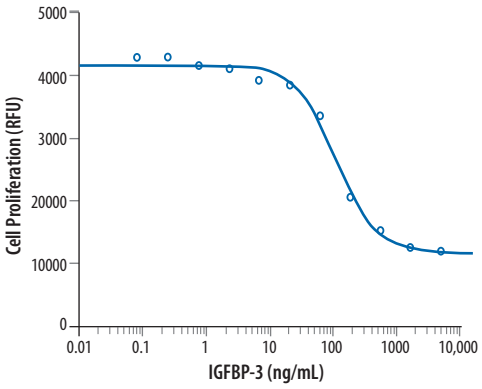
KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

XIII. Other Angiogenesis & Vasculogenesis Molecules

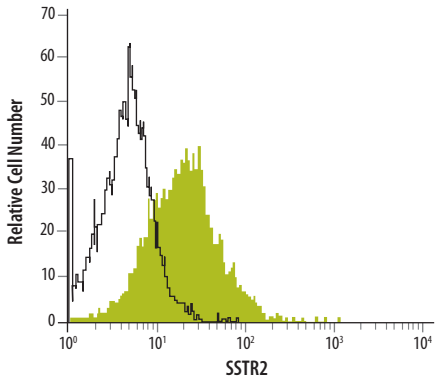
Several other cellular factors may also be involved in regulating angiogenesis, although the precise mechanisms by which they act are not currently well understood. The Insulin-like Growth Factor Binding Proteins (IGFBP1-6) modulate the biological activities of IGF proteins. IGF-I and –II induce proliferation, migration, and differentiation of a wide variety of cell types including endothelial cells, vascular smooth muscle cells, and cancer cells. The effects of IGFBPs on angiogenesis and tumorigenesis are now being investigated.

Another molecule that may also regulate angiogenesis is Heme Oxygenase 1 (HO-1), an enzyme that is induced during hypoxia and inflammation. Its involvement in angiogenesis was suggested by the findings that HO-1 promotes endothelial cell proliferation *in vitro*, and HO-1 antagonists can inhibit VEGF-induced endothelial cell activities. Many studies indicate that HO-1 has a protective function in damaged vascular tissue by promoting endothelial progenitor cell mobilization and non-inflammatory VEGF-induced angiogenesis. While HO-1 may have beneficial effects for cardiovascular diseases, it is also frequently upregulated in tumors and is associated with tumor progression.

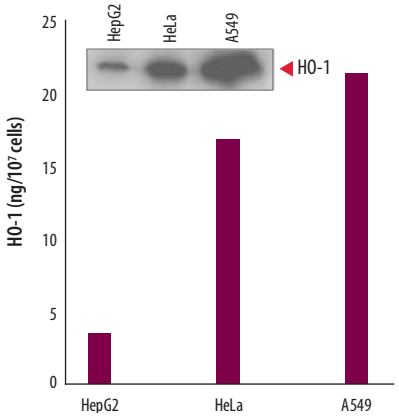
Somatostatin has been characterized as an anti-angiogenic molecule. It binds to one of five G protein-coupled Somatostain receptors (SSTR1-5) and has both anti-proliferative and anti-secretory effects. Somatostatin receptors are expressed on both normal and cancer cells in multiple organs. Their activation has been shown to inhibit hormone secretion and to promote cell cycle arrest or apoptosis. In addition, SSTRs mediate the release of cytokines and growth factors such as IGF-I, FGF basic, and VEGF, inhibit monocyte chemotaxis, and prevent tumor cell proliferation. Determining the molecular mechanisms by which somatostatin functions is currently under investigation.



IGFBP-3-Mediated Inhibition of IGF-II-Induced MCF-7 Cell Proliferation. MCF-7 human breast cancer cells, treated with 14 ng/mL recombinant human IGF-II (Catalog # 292-G2), were incubated with the indicated concentrations of recombinant human IGFBP-3 (Catalog #675-B3). Relative cell number was determined in a fluorometric assay using the redox sensitive dye, Resazurin (Catalog # AR002).

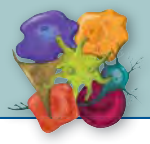


Detection of Somatostatin Receptor 2 by Flow Cytometry. MDA-MB-231 human breast cancer cells were incubated with anti-human Somatostatin Receptor 2 (SSTR2) monoclonal antibody (Catalog # MAB4224; filled histogram) or with mouse IgG_{2A} isotype control antibody (Catalog # MAB003; open histogram). This was followed by staining with APC-conjugated anti-mouse antibody (Catalog # F0101B).



Quantification of HO-1 Levels in Human Cancer Cell Lines using the DuoSet® IC ELISA. Lysates from HepG2 human hepatocellular liver carcinoma cells, HeLa human cervical epithelial carcinoma cells, and A549 human lung carcinoma cells were assayed using the human Total HO-1/HMOX1 DuoSet IC ELISA Development System (Catalog # DYC3776; bar graph). The same lysates were also immunoblotted using anti-human/mouse HO-1/HMOX polyclonal antibody (Catalog # AF3776; inset).

PRODUCTS FOR OTHER MOLECULES RELATED TO ANGIOGENESIS & VASCULOGENESIS LISTED ON PAGE 46>>>



PRODUCTS FOR OTHER MOLECULES RELATED TO ANGIOGENESIS & VASCULOGENESIS

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
AGE-BSA	B			
Angiogenesis Array Kits				H M
Angiogenin	H	H	H	H
BAI1		H		
BAI3		H		
BMP-2	H Z	H Z	H M R	
BMP-2/BMP-4		H		
BMP-2a	Z			
BMP-4	H M Z	H Z	H	
BMPR-IA/ALK-3	H M	H		
BMPR-IB/ALK-6	H M	H M		
BMPR-II	H	H		
BVES		H		
Chondromodulin-1		H		
COX-2		H M		
EG-VEGF/PK1	H M	H M R	H	
EDNRB/Endothelin R Type B		H		
Endothelin-1		H	H	
ENPP-2/Autotaxin	H	H		
Erythropoietin	H M R Ca	H M	H M R	
FGL2	M			
HO-1/HMOX1/HSP32		H M R	H	
IGFBP-1	H M	H M	H	
IGFBP-2	H M	H M	H M	
IGFBP-3	H M	H M	H M	
IGFBP-4	H	H	H	
IGFBP-5	H M	H M	H M	
IGFBP-6	H M	H M	H M	
IGFBP-rp1/IGFBP-7	H M	H M		

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAS	MULTIPLEX/ARRAY ASSAY KITS & REAGENTS
IGF-I	H M R	H M	H M	
IGF-II	H M	H M	M	
Neuregulin-1/NRG1	H	H	H	
NRG1-α/HRG1-α	H	H		
NRG1-β1/HRG1-β1	H	H	H	
NRG1 Isoform GGF2		H		
NRG1 Isoform SMDF	H	H		
Nitric Oxide			Ms	
eNOS		H	H	
iNOS		H	H	
nNOS		H		
Patched		M		
Patched 2		H		
PEDF R		H M R		
PGE2			Ms	
Prohibitin		H		
Prokineticin R1		H		
Prolactin	H M	H M	H M	
Proliferin		M		
Proliferin-related Protein/ Plfr		M		
Serpin B5/Maspin		H		
Serpin F1/PEDF		H M		
Somatostatin R2/SSTR2		H		
Somatostatin R5/SSTR5		H		
Sonic Hedgehog	H M	H M	M	
Vasohibin		H		
Vasostatin		H		

KEY: **H:** Human, **M:** Mouse, **R:** Rat, **B:** Bovine, **C:** Canine, **Ch:** Chicken, **D:** *Drosophilia*, **E:** Equine, **F:** Feline, **Ms:** Multi-species, **P:** Porcine, **Pr:** Primate, **Rb:** Rabbit, **X:** *Xenopus*, **Z:** Zebrafish

SPECIALIZED TOOLS FOR ANGIOGENESIS RESEARCH

I. Proteome Profiler™ Antibody Arrays

R&D Systems Proteome Profiler Arrays provide a rapid, sensitive method to simultaneously detect the relative levels of multiple analytes in a single sample, without using any specialized equipment. Each kit contains buffers, detection conjugates, and 2-8 nitrocellulose membranes spotted with a carefully selected panel of specific capture antibodies.

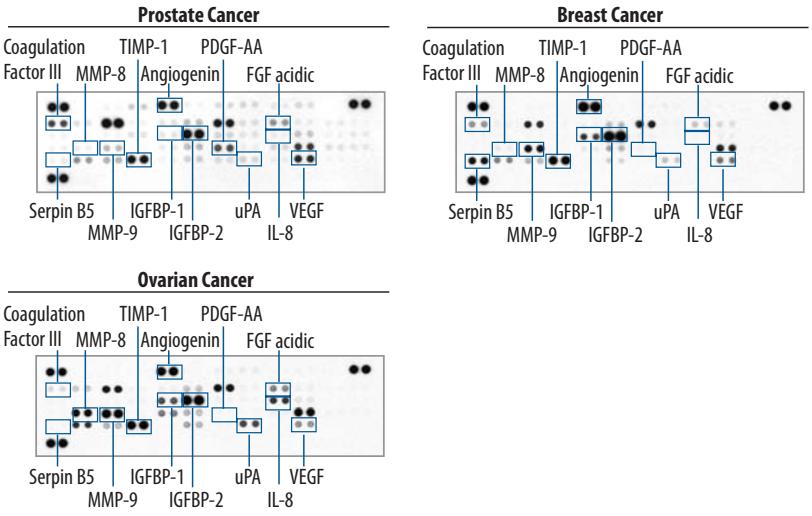
Two new arrays have recently been developed that are designed to detect multiple angiogenesis-related proteins. The Human (Catalog # ARY007) and Mouse (Catalog # ARY015) Angiogenesis Arrays include antibodies that detect soluble growth and differentiation factors, regulatory proteins, extracellular matrix components, and proteases. Other arrays are available for detecting apoptosis- or obesity-related proteins, cytokines, stem cell markers, or phosphorylated RTKs, immunoreceptors, or intracellular signaling factors. For more information, please visit our website at www.RnDSystems.com/go/ProteomeProfiler

Human Angiogenesis Array

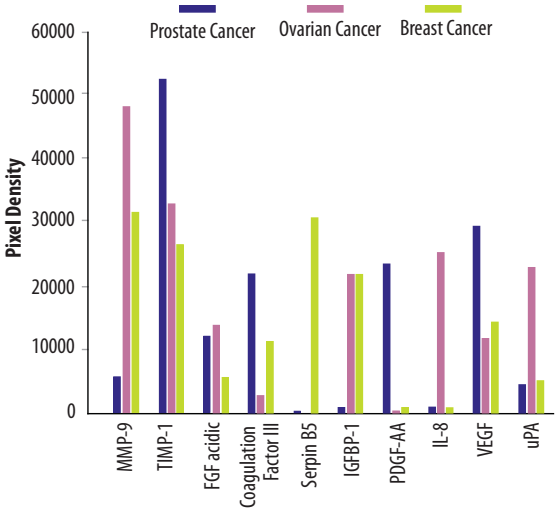
PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Angiogenesis Array Kit	Contains 4 arrays - each spotted in duplicate with 55 different angiogenesis antibodies	ARY007	1 Kit

Activin A, ADAMTS1, Angiogenin, Angiopoietin-1, Angiopoietin-2, Angiostatin/Plasminogen, Amphiregulin, Artemin, Coagulation Factor III/TF, CXCL16, DPPIV/CD26, EGF, EG-VEGF, Endoglin, Endostatin/Collagen XVIII, Endothelin-1, FGF acidic, FGF basic, FGF-4, FGF-7/KGF, GDNF, GM-CSF, HB-EGF, HGF, IGFBP-1, IGFBP-2, IGFBP-3, IL-1β, IL-8/CXCL8, LAP (TGF-β1), Leptin, MCP-1/CCL2, MIP-1α/CCL3, MMP-8, MMP-9, NRG1-β1/HRG1-β1, Pentraxin 3, PD-ECGF, PDGF-AA, PDGF-AB/BB, Persephin, PF4/CXCL4, P/GF, Prolactin, Serpin B5, Serpin E1/PAI-1, Serpin F1/PEDF, TIMP-1, TIMP-4, Thrombospondin-1, Thrombospondin-2, uPA, Vasohibin, VEGF, VEGF-C

A



B



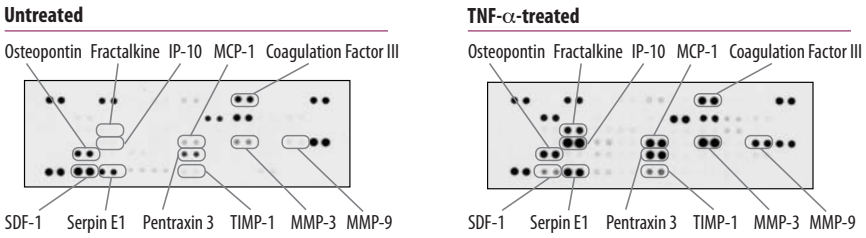
Detection of Angiogenesis-Related Proteins in Cancer Tissue Lysates using the Human Angiogenesis Array. A. The Proteome Profiler Human Angiogenesis Array (Catalog # ARY007) was used to profile angiogenesis-related proteins in lysates from human prostate, ovarian, and breast cancer tissues. **B.** Histogram profiles for select analytes were generated by quantifying the mean spot pixel density from the array membranes using image software analysis.

Mouse Angiogenesis Array

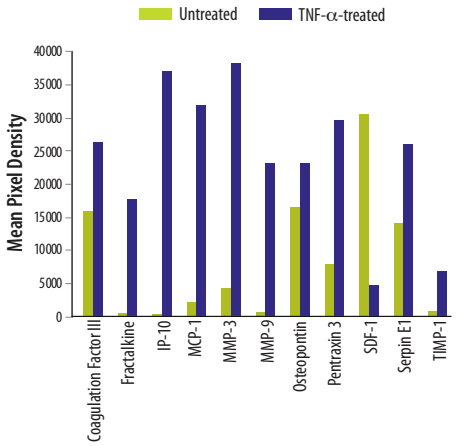
PRODUCT	DESCRIPTION	CATALOG #	SIZE
Mouse Angiogenesis Array Kit	Contains 4 arrays - each spotted in duplicate with 53 different angiogenesis antibodies	ARY015	1 Kit

ADAMTS1, Amphiregulin, Angiogenin, Angiopoietin-1, Angiopoietin-3, Coagulation Factor III/TF, CXCL16, Cyr61/CNN1, DLL4, DPPIV/CD26, EGF, Endoglin/CD105, Endostatin/Collagen XVIII, Endothelin-1, FGF acidic, FGF basic, FGF-7/KGF, Fractalkine, GM-CSF, HB-EGF, HGF, IGFBP-1, IGFBP-2, IGFBP-3, IL-1α, IL-1β, IL-10, IP-10/CRG-2/CXCL10, JE/MCP-1/CCL2, KC/CXCL1, Leptin, MIP-1α/CCL3, MMP-3 (pro and mature), MMP-8 (pro), MMP-9 (pro and active), NOV/CCN3/IGFBP-9, Osteopontin, PD-ECGF, PDGF-AA, PDGF-AB/BB, Pentraxin-3, PF4/CXCL4, PlGF-2, Prolactin, Proliferin, SDF-1/CXCL12, Serpin E1/PAI-1, Serpin F1/PEDF, Thrombospondin-2,TIMP-1, TIMP-4, VEGF, VEGF-B

A



B



Multiple Proteins in TNF-α-treated Balb/3T3 Cells were assessed using the Mouse Angiogenesis Array. A. The Proteome Profiler Mouse Angiogenesis Array Kit (Catalog # ARY015) was used to simultaneously assess the relative levels of 53 mouse angiogenesis-related proteins in cell lysates from Balb/3T3 mouse embryonic fibroblast cells that had been left untreated or treated with recombinant mouse TNF-α (Catalog # 410-MT) for 24 hours. **B.** Histogram profiles for select proteins were generated by quantifying the mean spot pixel density from the arrays using image software analysis. Green bars represent protein levels in lysates from untreated cells and purple bars represent protein levels in lysates from TNF-α-treated cells.

Human Apoptosis Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Apoptosis Array Kit	Contains 4 arrays - each spotted in duplicate with 35 different apoptosis-related antibodies	ARY009	1 Kit

Bad, Bax, Bcl-2, Bcl-x, Pro-Caspase-3, Cleaved Caspase-3, Catalase, Claspin, Clusterin, Cytochrome c, FADD, Fas/TNFSF6, HIF-1α, HO-1/HMOX1/HSP32, HO-2/HMOX2, HSP27, HSP60, HSP70, HTRA2/Omi, cIAP-1, cIAP-2, Livin, p21/CIP1/CDNK1A, p27/Kip1, PON2, Phospho-p53 (S15), Phospho-p53 (S46), Phospho-p53 (S392), Phospho-Rad17 (S635), SMAC/Diablo, Survivin, TNF RI/TNFRSF1A, TRAIL R1/DR4, TRAIL R2/DR5, XIAP

Human Cytokine Array, Panel A

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Cytokine Array Kit, Panel A	Contains 4 arrays - each spotted in duplicate with 36 different cytokine antibodies	ARY005	1 Kit

C5a, CD40 Ligand, G-CSF, GM-CSF, GROα, I-309, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-13, IL-16, IL-17, IL-17E, IL-23, IL-27, IL-32α, IP-10, I-TAC, MCP-1, MIF, MIP-1α, MIP-1β, RANTES, SDF-1, Serpin E1/PAI-1, TNF-α, TREM-1

Mouse Cytokine Array, Panel A

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Mouse Cytokine Array Kit, Panel A	Contains 4 arrays - each spotted in duplicate with 40 different cytokine antibodies	ARY006	1 Kit

BLC, C5a, G-CSF, GM-CSF, I-309, Eotaxin, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 p70, IL-13, IL-16, IL-17, IL-23, IL-27, IP-10, I-TAC, KC, M-CSF, JE, MCP-5, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, SDF-1, TARC, TIMP-1, TNF-α, TREM-1

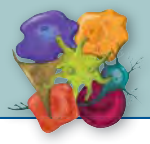
Rat Cytokine Array, Panel A

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Rat Cytokine Array Kit, Panel A	Contains 4 arrays - each spotted in duplicate with 29 different cytokine antibodies	ARY008	1 Kit

CINC-1, CINC-2α/β, CINC-3, CNTF, Fractalkine, GM-CSF, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-17, IP-10, LIX, MIG, MIP-1α, MIP-3α, RANTES, L-Selectin, Thymus Chemokine, TIMP-1, TNF-α, VEGF

Mouse Adipokine Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE



Proteome Profiler™ Antibody Arrays continued

Mouse Adipokine Array	Contains 4 membranes - each spotted in duplicate with 38 different obesity-related antibodies	ARY013	1 Kit
Adiponectin, AgRP, ANGPT-L3, CRP, DPPIV, Endocan, Fetuin A, FGF acidic, FGF-21, HGF, ICAM-1/CD54, IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IL-6, IL-10, IL-11, Leptin, LIF, Lipocalin-2/NGAL, MCP-1, M-CSF, Oncostatin M, Pentraxin 2, Pentraxin 3, Pref-1, RAGE, RANTES/CCL5, RBP4, Resistin, Serpin E1/PAI-1, TIMP-1, TNF-α, VEGF			

Human Phospho-Immunoreceptor Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Phospho-Immunoreceptor Array Kit	Contains 4 arrays - each spotted in duplicate with 59 different antibodies recognizing proteins involved in immunoreceptor signaling.	ARY004	1 Kit
2B4, BLAME, BTLA, CD3ε, CD5, CD6, CD28, CD84, CD229, CEACAM-1, CLEC-1, CLEC-2, CRACC, CTLA-4, DCIR, Dectin-1, DNAM-1, FcεRII, FcγRIIA/B, FcγRIIIA, FcRH1, FcRH2, FcRH4, FcRH5, ILT2, ILT3, ILT4, ILT5, ILT6, Integrin β3, KIR2DL4, LAIR-1, LAIR-2, LMIR1, LMIR2, LMIR3, LMIR6, MDL-1, NKp30, NKp44, NKp46, NKp80, NTB-A, PD-1, PECAM, SHIP-1, SHP-1, SHP-2, Siglec-2, Siglec-3, Siglec-5, Siglec-7, Siglec-9, Siglec-10, SIRP-β1, SLAM, TREM-1, TREM-2, TREML1			

Human Phospho-Kinase Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Phospho-Kinase Array Kit	Contains 4 sets of 2 membranes - each spotted in duplicate with 46 different kinase antibodies	ARY003	1 Kit
Akt (S473), Akt (T308), AMPKα1 (T174), AMPKα2 (T172), β-Catenin, Chk-2 (T68), CREB (S133), ERK1/2 (T202/Y204)/(T185/Y187), FAK (Y397), Fgr (Y412), Fyn (Y420), GSK-3α/β (S21/S9), Hck (Y411), HSP27 (S78/S82), JNK pan (T183/Y185)/(T221/Y223), c-Jun (S63), Lck (Y394), Lyn (Y397), MEK1/2 (S218/S222)/(S222/S226), MSK1/2 (S376/S360), eNOS (S1177), p27 (T198), p27 (T157), p38α (T180/Y182), p53 (S15), p53 (S46), p53 (S392), p70 S6 Kinase (T229), p70 S6 Kinase (T389), p70 S6 Kinase (T421/S424), Paxillin (Y118), PLCγ-1 (Y783), Pyk2 (Y402), RSK1/2 (S221), RSK1/2/3 (S380), Src (Y419), STAT1 (Y701), STAT2 (Y689), STAT3 (Y705), STAT4 (Y693), STAT5a (Y699), STAT5a/b (Y699), STAT5b (Y699), STAT6 (Y641), TOR (S2448), Yes (Y426)			

Human Phospho-Mitogen Activated Protein Kinase (MAPK) Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Phospho-MAPK Array Kit	Contains 4 arrays - each spotted in duplicate with 21 different antibodies recognizing MAPKs and other kinases	ARY002	1 Kit
Akt1 (S473), Akt2 (S474), Akt3 (S472), Akt pan (S473/S474/S472), ERK1 (T202/Y204), ERK2 (T185/Y187), GSK-3α/β (S21/S9), GSK-3β (S9), HSP27 (S78/S82), JNK1 (T183/Y185), JNK2 (T183/Y185), JNK3 (T221/Y223), JNK pan (T183/Y185)/(T221/Y223), MSK2 (S360), p38α (T180/Y182), p38β (T180/Y182), p38δ (T180/Y182), p38γ (T183/Y185), p70 S6 Kinase (T421/S424), RSK1 (S380), RSK2 (S386)			

Human Phospho-Receptor Tyrosine Kinase (RTK) Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Phospho-RTK Array Kit	Contains 4 arrays - each spotted in duplicate with 42 different RTK antibodies	ARY001	1 Kit
Axl, Dtk, EGF R, EphA1, EphA2, EphA3, EphA4, EphA6, EphA7, EphB1, EphB2, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R1, FGF R2α, FGF R3, FGF R4, Flt-3, HGF R, IGF-I R, Insulin R, M-CSF R, Mer, MSP R, MuSK, PDGF Rα, PDGF Rβ, c-Ret, ROR1, ROR2, SCF R, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1, VEGF R2, VEGF R3			

Mouse Phospho-Receptor Tyrosine Kinase (RTK) Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Mouse Phospho-RTK Array Kit	Contains 4 arrays - each spotted in duplicate with 39 different RTK antibodies	ARY014	1 Kit
Axl, Dtk, EGF R, EphA1, EphA2, EphA3, EphA6, EphA7, EphA8, EphB1, EphB2, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R2 (IIc), FGF R3, FGF R4, Flt-3, HGF R, IGF-I R, Insulin R, M-CSF R, Mer, MSP R, MuSK, PDGF Rα, PDGF Rβ, c-RET, SCF R, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1, VEGF R2, VEGF R3			

Human Pluripotent Stem Cell Array

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human Pluripotent Stem Cell Array Kit	Contains 8 arrays - each spotted in duplicate with 15 different stem cell marker antibodies	ARY010	1 Kit
E-Cadherin, α-Fetoprotein, GATA-4, Goosecoid, HCG, HNF-3β/FoxA2, Nanog, Oct-3/4, Otx2, PDX-1/IPF1, Snail, SOX2, SOX17, TP63/TP73L, VEGF R2/KDR/Flk-1			

II. Fluorokine® MAP Multiplex Kits for the Luminex® Platform

R&D Systems offers a choice of products for Fluorokine Multianalyte Profiling (FMAP) Multiplex Assays: as a stand-alone pre-mixed kit or as a Base Kit in combination with a panel of Analyte Kits.* Each Analyte Kit contains antibody-coated microparticles and biotin-conjugated detection antibodies. The Base Kit contains all of the other reagents needed to perform the assay. This arrangement allows the end-user maximum flexibility in creating panel composition. For ease of use, we also offer pre-mixed complete kits which contain all of the reagents necessary to simultaneously determine the concentrations of multiple molecules in a single sample.

Fluorokine MAP Multiplex Assays are subject to the same development, validation, manufacturing, and quality control standards as our Quantikine® ELISAs, the world's most referenced immunoassays. The result is accurate, sensitive, and reproducible multiplex assays. For each sample type, the panel is evaluated and validated for sensitivity, precision, recovery, sample linearity, specificity, and the ability to recognize both the natural and recombinant analyte.** Furthermore, all analytes in a given panel are optimized and tested together to ensure multiplex compatibility.

We now offer a new Human Angiogenesis Base Kit (Catalog # LAN000) and related analyte-specific bead sets for the simultaneous measurement of multiple angiogenesis-related proteins including VEGF, VEGF-D, PlGF, PDGF-AA, PDGF-BB, FGF acidic, FGF basic, Angiopoietin-1, Endostatin, Thrombospondin-2, and Angiogenin. Kits are also available for the simultaneous detection of multiple MMPs, TIMPs, adhesion proteins, obesity-related proteins, and cytokines.

For more information, please visit our website at www.RnDSystems.com/go/Multiplex

What you need to run a successful Fluorokine MAP experiment

1 Multiplex Panel Base Kit

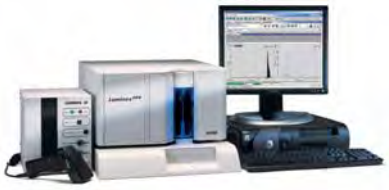
> Protein Standards
> Diluents
> Wash Buffer
> Streptavidin-PE
> Detailed protocol

2 Fluorokine MAP Bead Set(s)

Tailor your experiment by mixing & matching molecule-specific bead sets designed to work with your chosen base kit.

> Analyte-specific antibody-coated beads
> Biotinylated detection antibody concentrate

3 Luminex® Analyzer



> A dual laser, flow-based detection instrument
> For availability see our website at www.RnDSystems.com

Pre-mixed kits that include bead sets & all other reagents are available for some analytes; no need for the separate base kit.

*Analyte Kits are validated only for use with their designated Base Kit.

**For each sample type, a single, multipurpose diluent is used to optimize recovery, linearity and reproducibility. Such a multipurpose, single diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.

Fluorokine® MAP Multiplex Kits continued

Human Angiogenesis Panel A

BASE KIT	CONTENTS	CATALOG #
Human Angiogenesis Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluent, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LAN000

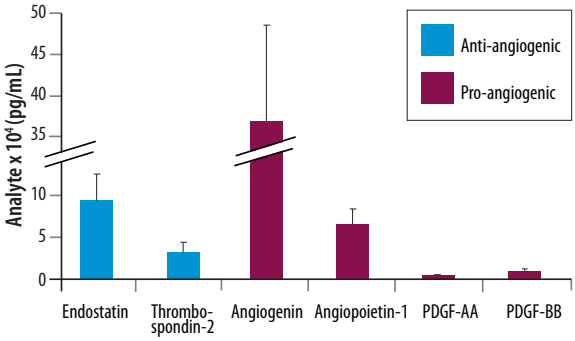
ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
PDGF-AA	C S P U M	1 pg/mL	LAN221
PDGF-BB	C S P U M	2 pg/mL	LAN220
PlGF	C S P U M	1 pg/mL	LAN264
VEGF	C S P U M	1.84 pg/mL	LUH293
VEGF-D	C S P U M	19.1 pg/mL	LAN622
FGF acidic	C S P U M	6.7 pg/mL	LAN232

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
FGF basic	C S P*** U M	4.91 pg/mL	LUH233
Angiopoietin-1	C S P U M	51.1 pg/mL	LAN923
Endostatin	C S P U M	31.9 pg/mL	LAN1098
Thrombospondin-2	C S P U M	57.4 pg/mL	LAN1635
Angiogenin	C S P U M	2.7 pg/mL	LAN265

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Cards included in the Base Kit.

***Not suitable with heparin plasma.



Simultaneous Measurement of Angiogenesis-related Biomarkers in Human Serum Samples. Protein levels of several angiogenesis-related proteins were simultaneously assessed in human serum samples using the Fluorokine MAP Angiogenesis Kit (Catalog # LAN000) and multiplexed analyte-specific bead sets. Data are presented as the mean ± standard deviation (n=15).

Human Adhesion Panel

BASE KIT	CONTENTS	CATALOG #
Human Adhesion Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LAD000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
sICAM-1/CD54	C S P	3.7 pg/mL	LAD720
sE-Selectin/CD62E	C S P	1.61 pg/mL	LAD724

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
sP-Selectin/CD62P	C S P	11.62 pg/mL	LAD137
sVCAM-1/CD106	C S P	8.21 pg/mL	LAD809

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

Human MMP Panel

BASE KIT	CONTENTS	CATALOG #
Human MMP Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LMP000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
MMP-1	C S P***	6.3 pg/mL	LMP901
MMP-2	C S P	38.9 pg/mL	LMP902
MMP-3	C S P	2.6 pg/mL	LMP513
MMP-7	C S P	29.5 pg/mL	LMP907

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
MMP-8	C S P	12.5 pg/mL	LMP908
MMP-9	C S P	11.0 pg/mL	LMP911
MMP-12	C S P	1.9 pg/mL	LMP919
MMP-13	C S	28.6 pg/mL	LMP511

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

***Not suitable with EDTA plasma

Human Obesity Panel

BASE KIT	CONTENTS	CATALOG #
Human Obesity Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LOB000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
Adiponectin/Acrp30	C S P	19.8 pg/mL.	LOB1065
C-Reactive Protein	C S P	1.9 pg/mL	LOB1707
CCL2/MCP-1	C S P	0.47 pg/mL	LUH279
Complement Factor D/Adipsin	C S P	9.5 pg/mL	LOB1824
IL-6	C S P	1.11 pg/mL	LUH206

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
IL-10	C S P	0.30 pg/mL	LUH217
Leptin	C S P	20.2 pg/mL	LUB398
Resistin	C S P	7.3 pg/mL	LOB1359
Serpin E1/PAI-1	C S P	0.29 pg/mL	LOB1786
TNF-α	C S P	1.50 pg/mL	LUH210

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

Fluorokine® MAP Multiplex Kits continued

Human Cytokine Panel A

BASE KIT	CONTENTS	CATALOG #
Human Panel A	Standard Cocktails, Microparticle Diluent, Calibrator Diluent, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LUH000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
CCL2/MCP-1	C S P	0.47 pg/mL	LUH279
CCL3/MIP-1α	C S P	1.45 pg/mL	LUH270
CCL4/MIP-1β	C S P	0.72 pg/mL	LUH271
CCL5/RANTES	C S P	1.91 pg/mL	LUH278
CXCL5/ENA-78	C S P	4.14 pg/mL	LUH254
CXCL8/IL-8	C S P	1.97 pg/mL	LUH208
FGF basic	C S P***	4.91 pg/mL	LUH233
G-CSF	C S P	1.48 pg/mL	LUH214
GM-CSF	C S P	1.98 pg/mL	LUH215C
IFN-γ	C S P	1.27 pg/mL	LUH285
IL-1α/IL-1F1	C S P	0.36 pg/mL	LUH200B

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
IL-1β/IL-1F2	C S P	0.57 pg/mL	LUH201
IL-1ra/IL-1F3	C S P	5.23 pg/mL	LUH280
IL-2	C S P	2.23 pg/mL	LUH202
IL-4	C S P	4.46 pg/mL	LUH204
IL-5	C S P	0.71 pg/mL	LUH205
IL-6	C S P	1.11 pg/mL	LUH206
IL-10	C S P	0.30 pg/mL	LUH217
IL-17	C S P	1.10 pg/mL	LUH317
TNF-α/TNFSF1A	C S P	1.50 pg/mL	LUH210
Tpo	C S P	9.94 pg/mL	LUH288
VEGF	C S P	1.84 pg/mL	LUH293

*Analyte Kits are validated only for use with their designated Base Kit. Human Panel A controls are also available, please inquire.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Cards included in the Base Kit.

***Not suitable with heparin plasma

Human Cytokine Panel B

BASE KIT	CONTENTS	CATALOG #
Human Panel B	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LUB000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
CCL11/Eotaxin	C S P	8.2 pg/mL	LUB320
CD40 Ligand/TNFSF5	C S P	23.4 pg/mL	LUB201
CXCL10/IP-10	C P	0.10 pg/mL	LUB266
CXCL11/I-TAC	C S P	22.5 pg/mL	LUB672
EGF	C S P	1.58 pg/mL	LUB236

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
HGF	C S P	4.1 pg/mL	LUB294
IL-12 p70	C S P	14.9 pg/mL	LUB219
IL-13	C S P	15.6 pg/mL	LUB213
Leptin	C S P	20.2 pg/mL	LUB398

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

Mouse Cytokine Panel

BASE KIT	CONTENTS	CATALOG #
Mouse Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LUM000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
CCL2/JE/MCP-1	C S P	37.1 pg/mL	LUM479
CXCL1/KC	C S P	2.69 pg/mL	LUM453
CXCL2/MIP-2	C S P	10.1 pg/mL	LUM452
GM-CSF	C S P	3.29 pg/mL	LUM415B
IFN-γ	C S P	7.6 pg/mL	LUM485
IL-1β/IL-1F2	C S P	7.82 pg/mL	LUM401
IL-2	C S P	6.3 pg/mL	LUM402
IL-4	C	4.27 pg/mL	LUM404

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
IL-5	C S P	4.18 pg/mL	LUM405
IL-6	C S P	1.06 pg/mL	LUM406
IL-10	C S P	1.28 pg/mL	LUM417
IL-12 p70	C S P	22.42 pg/mL	LUM419
IL-13	C S P	42.0 pg/mL	LUM413
IL-17	C S P	10.8 pg/mL	LUM421
TNF-α/TNFSF1A	C S P	1.39 pg/mL	LUM410
VEGF	C S P	10.9 pg/mL	LUM493

*Analyte Kits are validated only for use with their designated Base Kit. Mouse Panel controls are also available, please inquire.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

Rat Cytokine Panel

BASE KIT	CONTENTS	CATALOG #
Rat Panel	Standard Cocktail, Microparticle Diluent, Calibrator Diluents, Wash Buffer Concentrate, Filter-bottomed Microplate, Mixing Bottles, Plate Sealers, Biotin Antibody Diluent, Streptavidin-PE	LUR000

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
CINC-2α/β	C S P	3.59 pg/mL	LUR516
CINC-3	C S P	8.44 pg/mL	LUR525
GM-CSF	C S P	3.13 pg/mL	LUR518
ICAM-1/CD54	C S P	3.40 pg/mL	LUR583
IFN-γ	C S P	137 pg/mL	LUR585
IL-13	C S P	6.99 pg/mL	LUR1945
IL-1α/IL-1F1	C S P	20.99 pg/mL	LUR500
IL-1β/IL-1F2	C S P	16.6 pg/mL	LUR501
IL-2	C S P	8.72 pg/mL	LUR502

ANALYTE KIT*	SAMPLE TYPE	SENSITIVITY**	CATALOG #
IL-4	C S P	1.84 pg/mL	LUR504
IL-6	C S P	71.3 pg/mL	LUR506
IL-10	C S P	11.6 pg/mL	LUR522
IL-18/IL-1F4	C S P	10.8 pg/mL	LUR521
L-Selectin/CD62L	C S P	51.9 pg/mL	LUR1534
TIMP-1	C S P	6.5 pg/mL	LUR580
TNF-α/TNFSF1A	C S P	19.5 pg/mL	LUR510
VEGF	C S P	3.51 pg/mL	LUR564

*Analyte Kits are validated only for use with their designated Base Kit.

**Sensitivity values listed here are representative values and can vary by lot. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

Pre-mixed Fluorokine MAP Multiplex Kits

PRODUCT	DESCRIPTION	CATALOG #	SIZE
Human TIMP 4-plex Kit	For the measurement of TIMP-1, -2, -3 and -4 in a single sample	LKT003	1 Kit
Multi-species TGF-β3-plex Kit	For the measurement of TGF-β1, -β2, and -β3 in a single sample	LKT001	1 Kit

III. Extracellular Matrix-Related Products

Extracellular Matrix Protein Pre-coated Plates for Adhesion Assays

The extracellular matrix (ECM) is made up of proteins that include collagen, fibronectin, laminin, and vitronectin. These molecules interact with cells via integrins and cell surface receptors, facilitating the adhesion of cells to the ECM. The resulting focal adhesions or focal contacts are important for the maintenance of tissue architecture and for supporting a variety of cellular processes including cell spreading, migration, proliferation, and differentiation during embryogenesis, wound healing, and tumor development. For more information on R&D Systems ECM-related Adhesion and Invasion Assays, please visit our website at www.RnDSystems.com/go/CellCulture

PRODUCT	DESCRIPTION	CATALOG #	SIZE*
Fibronectin Coated 96-well microplates	Bovine	CWP002	1 Pack
Fibronectin Coated 96-well microplates	Human	CWP001	1 Pack
Vitronectin Coated 96-well microplates	Bovine	CWP004	1 Pack
Vitronectin Coated 96-well microplates	Human	CWP003	1 Pack

*Each pack contains 5 microplates.

Basement Membrane Extracts

Cultrex® Basement Membrane Extract (BME) is a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) tumor that gels at 37°C to form a reconstituted basement membrane. It consists of laminin I, type IV collagen, entactin, and heparan sulfate proteoglycan. Cultrex BME can be used to promote and maintain the differentiated phenotype of multiple cell types including endothelial and smooth muscle cells. It is available with or without phenol red and in regular or reduced growth factor formulations.

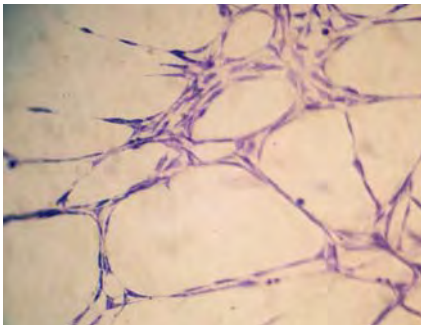
Rigorous Quality Control Standards Ensure:

- High Concentration - supports *in vivo* angiogenesis and tumorigenicity assays
- Cell Culture Qualified - no bacterial or fungal growth after 14 days at 37°C, no mycoplasma contamination, and endotoxin levels < 20 EU/mL
- Gel Formation Verified - gels quickly and maintains this form with culture medium for a period of at least 14 days at 37°C
- *In Vitro* Assays - promotes differentiation of endothelial cells into capillary-like structures and supports angiogenesis in a rat aortic ring assay

PRODUCT	CATALOG #	SIZE
Cultrex 3D Culture Matrix Basement Membrane Extract	3445-048-01	15 mL
Cultrex 3D Culture Matrix Rat Collagen I	3447-020-01	100 mg
Cultrex 3D Culture Matrix Laminin I	3446-005-01	5 mL
Cultrex Basement Membrane Extract	3432-005-01	5 mL
Cultrex Basement Membrane Extract - Phenol Red	3430-005-01	5 mL
Cultrex Basement Membrane Extract Reduced Growth Factor	3433-005-01	5 mL
Cultrex Basement Membrane Extract Reduced Growth Factor - Phenol Red	3431-005-01	5 mL

PRODUCT	CATALOG #	SIZE
Cultrex PathClear® BME	3432-005-02	5 mL
Cultrex PathClear BME - Phenol Red	3430-005-02	5 mL
Cultrex PathClear BME Reduced Growth Factor	3433-005-02	5 mL
Cultrex PathClear BME Reduced Growth Factor - Phenol Red	3431-005-02	5 mL
Cultrex HC20+ PathClear BME High Protein Concentration Basement Membrane Extract	3444-005-02	5 mL

A



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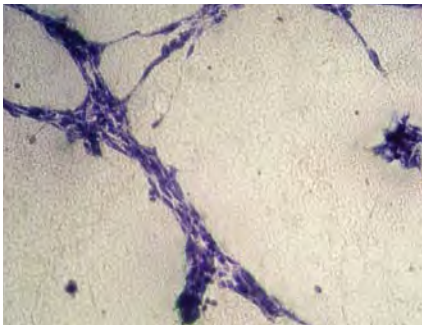


A. Rat Aortic Ring Assay using Cultrex Basement Membrane Extract and FBS. Rat aorta was incubated with Cultrex Basement Membrane Extract and fetal bovine serum (FBS) for 6 days at 37°C. New capillary formation was visualized.

B. Capillary Tube Formation using Cultrex Basement Membrane Extract and FBS. SVEC4-10 cells were incubated with Cultrex Basement Membrane Extract and fetal bovine serum (FBS) for 24 hours at 37°C. Capillary-like structures formed within 4 hours, and remained after 24 hours in the presence of FBS.

Cell Invasion Assays

Cultrex Cell Invasion Assays are ideally suited for the evaluation of compounds that influence cell migration through extracellular matrices. The assay utilizes a disposable Boyden chamber that offers several advantages: low start-up expense, flexible, standardized, high-throughput format, and no decontamination to remove residual factors.

PRODUCT	DESCRIPTION	CATALOG #	SIZE
BME Cell Invasion Assay	96-well assay for investigating chemotaxis, cell migration, and/or cell invasion for adhesive cell types.	3455-096-K	1 Kit
Collagen I Cell Invasion Assay	96-well assay for investigating chemotaxis, cell migration, and/or cell invasion for adhesive cell types.	3457-096-K	1 Kit
Collagen IV Cell Invasion Assay	96-well assay for investigating chemotaxis, cell migration, and/or cell invasion for adhesive cell types.	3458-096-K	1 Kit
Laminin I Cell Invasion Assay	96-well assay for investigating chemotaxis, cell migration, and/or cell invasion for adhesive cell types.	3456-096-K	1 Kit

Invasion Assay Schematic

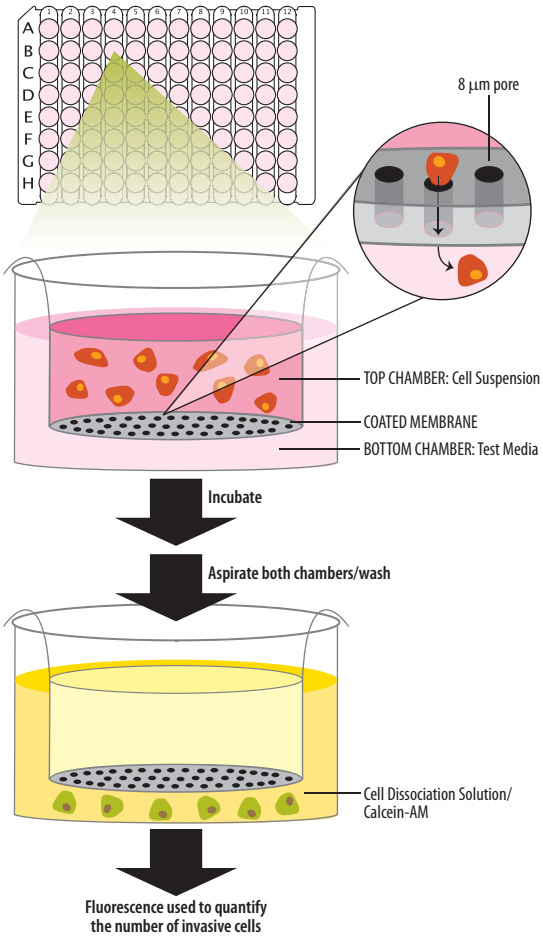
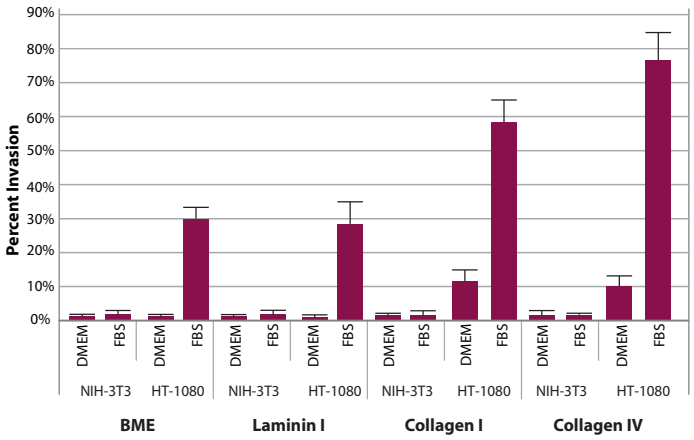


Illustration of a Cell Invasion Assay. The invasion chamber consists of two chambers separated by a filter coated with BME or different ECM components. The cell suspension is placed in the top chamber and incubated in the presence of test media containing specific chemoattractants in the bottom chamber. Cells migrate from the top chamber through the coated filter pores to the bottom of the filter. Cell dissociation/Calcein-AM solution is placed in the bottom chamber to dissociate the migrating cells from the filter and add a fluorescent label. Fluorescence in the bottom chamber is proportional to the number of migrating cells.



Quantification of Cell Invasion. Cultrex Cell Invasion Assay Kits (Catalog # 3455-096-K, # 3456-096-K, # 3457-096-K, # 3458-096-K) were used to quantify the ability of 10% FBS to stimulate the migration of fibroblastic cell lines on different extracellular matrix components. Results from four experiments were quantified for both non-invasive (NIH-3T3) and invasive (HT-1080) cell types.

For information on other ECM products for angiogenesis research available from R&D Systems, please see the product listings in the *Extracellular Matrix-related Molecules* section of this catalog.

IV. PlusCellect™ Cell Selection & Detection Kits

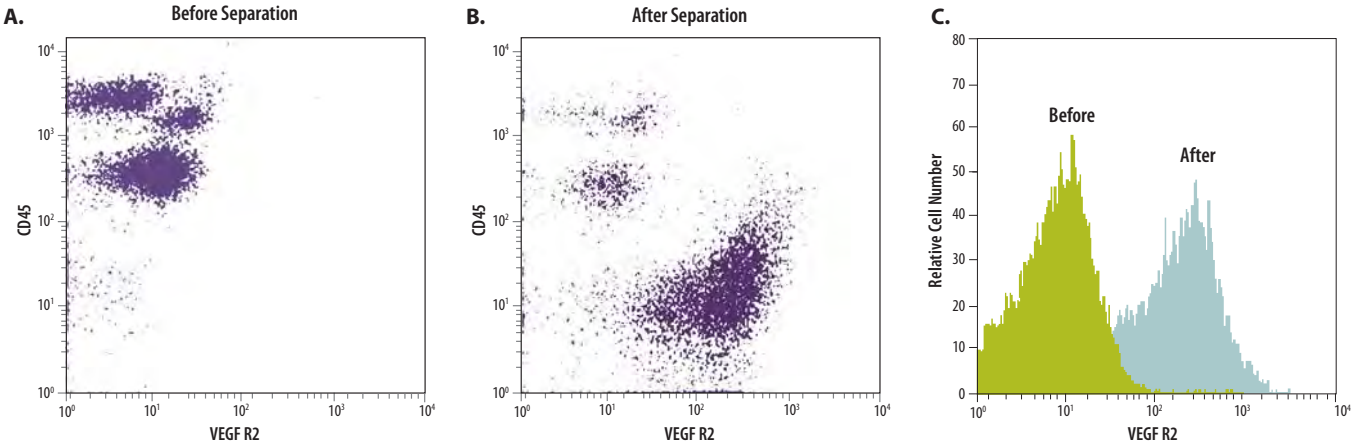
R&D Systems PlusCellect line offers a simple and efficient method for positive selection (or depletion) of rare or less abundant cell populations. These innovative kits include two different antibodies: one for isolation of the desired cell population and a second for detection of the intended target cells following purification. The inclusion of two antibodies facilitates the direct assessment of the enrichment efficiency. PlusCellect Kits can be used in combination with MagCellect™ Streptavidin beads or any other compatible magnetic system (beads and magnet). Several PlusCellect Kits are available to simplify the isolation of endothelial cells, cancer cells, or stem cell progenitors using specific cell surface markers. For more information, please visit our website at www.RnDSystems.com/go/PlusCellect

PlusCellect Cell Selection & Detection Kits

ANALYTE KIT	RESEARCH INTEREST*	PROCESSING CAPACITY	CAT. #	SIZE
Human ACE/CD143	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS929	1 Kit
Human E-Cadherin	■ ■	25 samples or up to 1 x 10^9 cells	PLS1838	1 Kit
Mouse E-Cadherin	■ ■	20 samples or up to 1 x 10^9 cells	PLS5748	1 Kit
Human VE-Cadherin	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS938	1 Kit
Human CCR5	■	25 samples or up to 1 x 10^9 cells	PLS180	1 Kit
Human CD4	■	25 samples or up to 1 x 10^9 cells	PLS379	1 Kit
Human CD31/PECAM-1	■ ■	25 samples or up to 1 x 10^9 cells	PLS806	1 Kit
Human CD44	■ ■	25 samples or up to 1 x 10^9 cells	PLS4948	1 Kit
Human CD45	■	25 samples or up to 1 x 10^9 cells	PLS1430	1 Kit
Human CXCR4	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS170	1 Kit
Human EGF R/ErbB1	■	25 samples or up to 1 x 10^9 cells	PLS1095	1 Kit
Human Endoglin/CD105	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS1097	1 Kit
Human EpCAM/TROP-1	■	25 samples or up to 1 x 10^9 cells	PLS960	1 Kit

ANALYTE KIT	RESEARCH INTEREST*	PROCESSING CAPACITY	CAT. #	SIZE
Human ErbB2/HER2	■	25 samples or up to 1 x 10^9 cells	PLS1129	1 Kit
Human HGF R/c-MET	■	25 samples or up to 1 x 10^9 cells	PLS358	1 Kit
Human Integrin V/CD51	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS1219	1 Kit
Human MCAM/CD146	■ ■	25 samples or up to 1 x 10^9 cells	PLS932	1 Kit
Human NCAM-1/CD56	■	25 samples or up to 1 x 10^9 cells	PLS2408	1 Kit
Human Nectin-4	■	25 samples or up to 1 x 10^9 cells	PLS2659	1 Kit
Human Podocalyxin	■	25 samples or up to 1 x 10^9 cells	PLS1658	1 Kit
Mouse Sca-1/Ly6	■	20 samples or up to 1 x 10^9 cells	PLS1226	1 Kit
Human E-Selectin/CD62E	■ ■	25 samples or up to 1 x 10^9 cells	PLS724	1 Kit
Human Tie-2	■ ■	25 samples or up to 1 x 10^9 cells	PLS313	1 Kit
Human VCAM-1/CD106	■ ■	25 samples or up to 1 x 10^9 cells	PLS809	1 Kit
Human VEGF R2/KDR/Flk-1	■ ■ ■	25 samples or up to 1 x 10^9 cells	PLS357	1 Kit

*Key: ■ Vascular Biology ■ Cancer ■ Stem Cells/Progenitors ■ Immunology



Isolation of VEGF R2⁺ Cells using the Human VEGF R2/KDR/Flk-1 PlusCellect Kit. The Human VEGF R2 PlusCellect Kit (Catalog # PLS357) was used to isolate VEGF R2⁺ cells from peripheral white blood cells using positive selection. Cells were stained with PE-conjugated anti-human VEGF R2 detection antibody (provided in the kit) before **(A)** and after **(B)** selection, and with APC-conjugated anti-human CD45 antibody (Catalog # FAB1430A) to detect contaminating blood cells. A histogram depicting data from the VEGF R2⁺ staining in **(A)** and **(B)** is also shown **(C).**

V. Apoptosis Detection Reagents

R&D Systems offers an extensive range of kits and reagents for the detection of apoptosis in a variety of different cell and tissue types. In addition to cell viability and proliferation assays, kits are available for detecting cell membrane changes, disruption of the mitochondrial membrane potential, activation of intracellular caspases, PARP activity, and DNA fragmentation (CometAssay®, TUNEL Kits, and DNA Laddering Kits). Several tissue-type specific TUNEL labeling kits are specialized for use with vascular tissue, cardiac tissue, or tumor samples. For more information on these kits, please visit our website at www.RnDSystems.com/go/ApoptosisDetectionKits

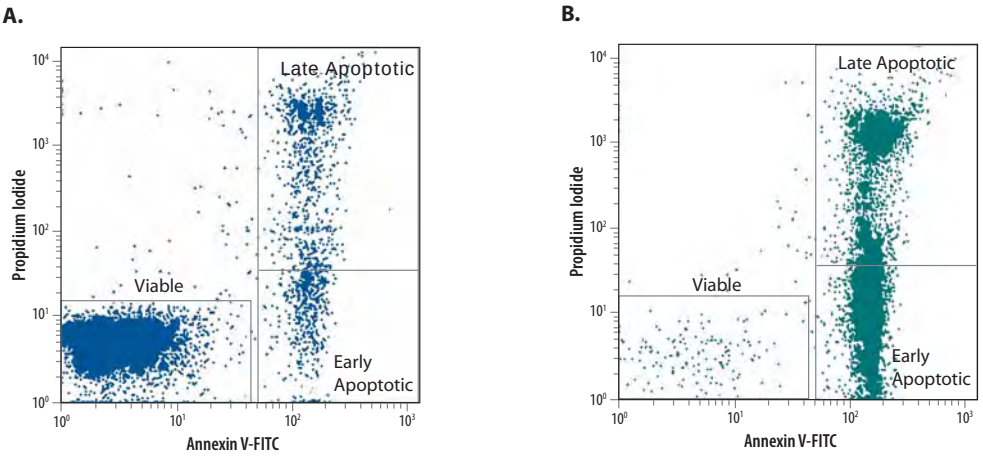
Cell Viability and Proliferation Assays

PRODUCT	DESCRIPTION	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
TACS™ MTT Assay	For the measurement of cell proliferation based on the reduction of the tetrazolium salt, MTT in metabolically active cells.	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT)	• Microplate reader (Absorbance)	• Unfixed cells	4890-025-K 4890-050-K	2500 Tests 5000 Tests
TACS XTT Assay	For the measurement of cell proliferation based on the reduction of the tetrazolium salt, XTT in metabolically active cells.	2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT)	• Microplate reader (Absorbance)	• Unfixed cells	4891-025-K	2500 Tests
Calcein AM Cell Viability Assay	For the measurement of cell viability based on the hydrolysis of Calcein AM to Calcein, a hydrophilic, fluorescent compound that is retained in the cytoplasm of cells with intact membranes.	Calcein	• Microplate reader (Fluorescence)	• Unfixed cells	4892-010-K	1000 Tests
Resazurin	A redox-sensitive dye that changes from a blue/non-fluorescent state to a pink/highly fluorescent state upon reduction to resorufin in viable cells.	Resorufin	• Microplate reader (Absorbance or Fluorescence)	• Unfixed cells	AR002	100 mL

Annexin V Assays

PRODUCT	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
TACS Annexin V -Biotin *in situ*	Streptavidin-Fluorochrome Conjugate (not provided)	• Flow Cytometry • Fluorescence microscopy	• Fixed Cells*	4835-01-K	100 Tests
TACS Annexin V -Fluorescein *in situ*	Fluorescein	• Flow Cytometry • Fluorescence microscopy	• Unfixed cells	4830-01-K 4830-250-K	100 Tests 250 Tests

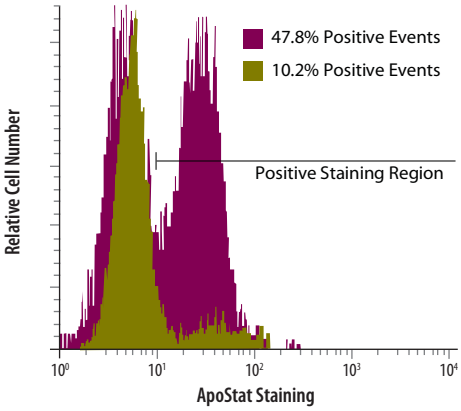
**Cells are fixed after labeling with Annexin V-Biotin.*



Detection of Apoptotic Dexamethasone-treated Thymocytes by Annexin V Staining. Thymocytes were left untreated **(A)** or treated with dexamethasone (100 nM) for 15.5 hours **(B)** and then stained using Annexin V-FITC and propidium iodide provided in the Annexin V-FITC Apoptosis Detection Kit (Catalog # 4830-01-K). The combination of Annexin V-FITC and propidium iodide allows for the differentiation between early apoptotic cells (Annexin V-FITC positive), late apoptotic and/or necrotic cells (Annexin V-FITC and propidium iodide positive) and viable cells (unstained). Analysis courtesy of Dr. C.M. Knudsen, Howard Hughes Medical Institute, St. Louis, MO.

ApoStat Apoptosis Detection Kit

PRODUCT	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
ApoStat Assay FITC-VD-FMK	FITC	• Flow Cytometry	• Unfixed cells	FMK012	100 Tests



Detection of Caspase Activation in Jurkat T Leukemic Cells. Jurkat human T leukemic cells were cultured in the absence (green histogram) or presence (purple histogram) of staurosporine (1 µM) for 3.5 hours and then stained with ApoStat (Catalog # FMK012) for 30 minutes. The cells were harvested, washed, and assayed by flow cytometry to determine the percentage of cells expressing active caspases.

DePsipher™ Mitochondrial Membrane Potential Assay

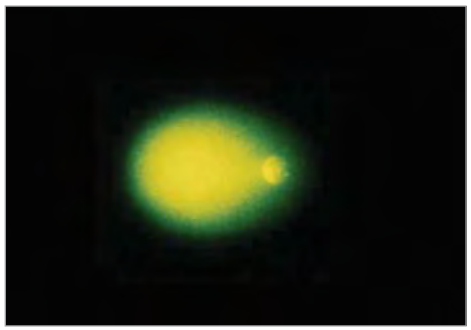
PRODUCT	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
DePsipher Assay	Lipophilic cation	• Flow Cytometry • Fluorescence microscopy	• Unfixed cells	6300-100-K	100 Tests

PARP Activity Assay

PRODUCT	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
PARP Universal Colorimetric Assay	TACS-Sapphire™	• Colorimetric Assay	• Unfixed cells • Fresh tissues	4677-096-K	96 Tests

Single-Cell Gel Electrophoresis Assay/Comet Assay™

PRODUCT	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
CometAssay® – Silver	Silver	• Light microscopy	• Unfixed cells • Fresh tissues	4251-050-K	50 Tests
CometAssay – SYBR®	SYBR Green I	• Fluorescence microscopy	• Unfixed cells • Fresh tissues	4250-050-K	50 Tests



Visualization of DNA Damage using the CometAssay – SYBR Kit. DNA fragmentation associated with oxidative DNA damage in WEH17.1 murine lymphoma cells was visualized using the CometAssay – SYBR Kit (Catalog # 4250-050-K). Following fixation of the cells, the slides were dried and stained following the instructions provided in the kit, and the cells were analyzed by microscopy. Undamaged DNA does not migrate far from the origin, while damaged DNA appears as a fluorescent "comet tail".

TUNEL Labeling Kits

Light & Electron Microscope-based Kits

PRODUCT	DESCRIPTION	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
TACS® TdT *In situ* Apoptosis Detection Kit - DAB	A complete kit supplied with the core reagents necessary for the TUNEL reaction and Streptavidin-HRP, DAB, and Methyl Green counterstain.	DAB	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4810-30-K	30 Tests
TACS TdT *In situ* Apoptosis Detection Kit- Blue Label	A complete kit supplied with the core reagents necessary for the TUNEL reaction and Streptavidin-HRP, TACS Blue Label™, and Nuclear Fast Red counterstain	TACS Blue Label	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4811-30-K	30 Tests
TACS TdT *In situ* Apoptosis Detection Kit- Core Reagents	Provides the core reagents necessary for the TUNEL reaction. Includes 10X TdT Labeling Buffer, TdT-dNTP Mix, TdT Enzyme, 10X TdT Stop Buffer, Streptavidin-HRP, 50X Co^{2+}, 50X Mg^{2+}, and 50X Mn^{2+}.	HRP-based Substrate System (not provided)	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4810-30-CK	30 Tests
TACS TdT *In situ* Apoptosis Detection Kit- Replenisher	Provides limiting core reagents necessary for the TUNEL reaction. Includes Proteinase K Solution, TdT-dNTP Mix, TdT Enzyme, Streptavidin-HRP, 50X Co^{2+}, 50X Mg^{2+}, and 50X Mn^{2+}.	HRP-based Substrate System (not provided)	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4810-30-R	30 Tests
TACS•XL® *In situ* Apoptosis Detection Kit - Basic	Provides the core reagents necessary for the TUNEL reaction. Includes Cytonin™, Proteinase K Solution, 10X TdT Labeling Buffer, 10X TdT Stop Buffer, B-dNTP Mix, TdT Enzyme, Streptavidin-HRP, Streptavidin Diluent, and Anti-BrdU antibody	HRP-based Substrate System (not provided)	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4828-30-K	30 Tests
TACS•XL *In situ* Apoptosis Detection Kit - DAB	A complete kit supplied with the core reagents necessary for the TUNEL reaction and biotin conjugated anti-BrdU, Streptavidin-HRP, DAB, and Methyl Green counterstain.	DAB	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4828-30-DK	30 Tests
TACS•XL *In situ* Apoptosis Detection Kit - Blue Label	A complete kit supplied with the core reagents necessary for the TUNEL reaction and biotin conjugated anti-BrdU, Streptavidin-HRP, TACS Blue Label, and Nuclear Fast Red counterstain.	TACS Blue Label	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4828-30-BK	30 Tests
TACS•XL *In situ* Apoptosis Detection Kit - Replenisher	Provides limiting core reagents necessary for the TUNEL reaction. Includes Proteinase K Solution, B-dNTP Mix, TdT Enzyme, Streptavidin-HRP, Streptavidin Diluent, and Anti-BrdU antibody.	HRP-based Substrate System (not provided)	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4828-30-R	30 Tests

Fluorescence & Flow Cytometry-based Kits

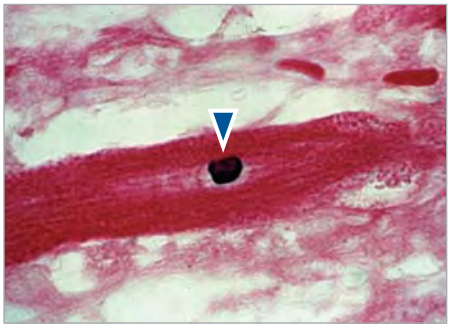
PRODUCT	DESCRIPTION	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
TACS TdT *In situ* Apoptosis Detection Kit - Fluorescein	A complete kit supplied with the core reagents necessary for the TUNEL reaction and Streptavidin-Fluorescein for the detection of apoptosis.	Fluorescein	• Fluorescence microscopy • Flow Cytometry	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4812-30-K	30 Tests
FlowTACS™ *In situ* Apoptosis Detection Kit	A complete kit that comes with the core reagents necessary for the TUNEL reaction and Streptavidin-Fluorescein for analysis in Flow Cytometry.	Fluorescein	• Fluorescence microscopy • Flow Cytometry	• Fixed cells	4817-60-K	60 Tests

Microplate Reader-Based Kits

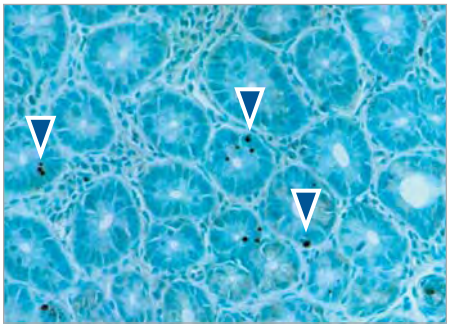
PRODUCT	DESCRIPTION	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
TiterTACS™ *In situ* Apoptosis Detection Kit	A complete kit supplied with the core reagents necessary for the TUNEL reaction and TACS-Sapphire optimized for colorimetric detection in 96-well plates.	HRP	• Microplate reader	• Suspension or monolayer cell cultures	4822-96-K	96 Tests

Tissue Type-Specific Colorimetric Kits

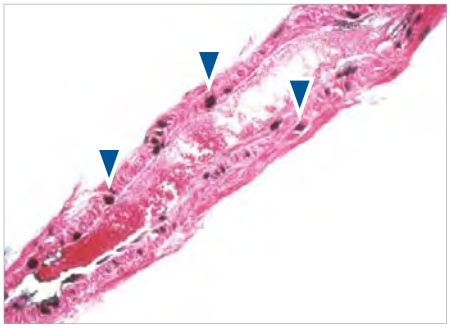
PRODUCT	DESCRIPTION	LABEL	TESTING FORMAT	SAMPLE TYPE	CATALOG #	SIZE
CardioTACS™ *In situ* Apoptosis Detection Kit	A complete kit supplied with the core reagents necessary for the TUNEL reaction and TACS Blue Label with Nuclear Fast Red counterstain optimized for labeling samples and generating positive controls in cardiac tissues.	TACS Blue Label	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4827-30-K	30 Tests
TumorTACS™ *In situ* Apoptosis Detection Kit	A complete kit supplied with the core reagents necessary for the TUNEL reaction and DAB Methyl Green counterstain optimized for labeling samples and generating positive controls in tumor samples	DAB	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4815-30-K	30 Tests
VasoTACS™ *In situ* Apoptosis Detection Kit	A complete kit supplied with the core reagents necessary for the TUNEL reaction and TACS Blue Label with Red counterstain C optimized for labeling samples and generating positive controls in vascular samples.	TACS Blue Label	• Light microscopy • Double labeling • Electron microscopy	• Fixed cells • Frozen tissues • Paraffin-embedded cells and tissues • Resin-embedded cells and tissues	4826-30-K	30 Tests



Visualization of an Apoptotic Rat Cardiac Myocyte using the CardioTACS *In situ* Detection Kit. Rat heart tissue was fixed in 4% formaldehyde overnight, and then paraffin-embedded. Five micron sections were prepared and placed onto glass microscope slides. Samples were processed following the CardioTACS Kit protocol (Catalog # 4827-30-K) and then visualized by light microscopy. An apoptotic cardiac myocyte is indicated by the arrow. Photo was provided courtesy of Dr. J. Zhang, FDA.



Apoptotic Cells within a Mouse Mammary Tumor Identified using the TumorTACS Kit. Mammary tumor was fixed in 4% paraformaldehyde overnight, and then paraffin-embedded. Five micron sections were prepared and placed onto glass microscope slides. The sample was processed following the TumorTACS Kit protocol (Catalog # 4815-30-K). Brown-stained nuclei indicate apoptotic cells (arrows).



Visualization of Apoptotic Cells in Vascular Tissue. Tissue from the small artery of a rat was formalin-fixed and paraffin-embedded following the initiation of drug-induced apoptosis. The tissue was processed according to the protocol in the VasoTACS Kit protocol (Catalog # 4826-30-K) and visualized by light microscopy. Brown-stained nuclei indicate apoptotic cells (arrows). Data was provided courtesy of Dr. J. Zhang, FDA.

notes

R&D SYSTEMS, INC. TOOLS FOR CELL BIOLOGY RESEARCH™

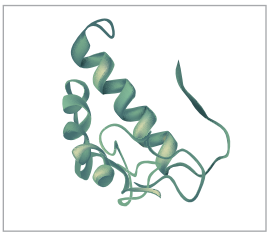
About Us

R&D Systems is a leading supplier of cell biology reagents with over 30 years of experience manufacturing specialty biological products that serve the basic research, clinical research, and clinical diagnostic markets. We offer products for the study of cancer, developmental biology, endocrinology, glycobiology, immunology, neuroscience, proteases, signal transduction, and stem cell research.

Recombinant & Natural Proteins

More than 1,700 recombinant and natural proteins from 15 different species are available from R&D Systems. Stringent production and purification standards, as well as rigorous bioassay or functional ELISA activity testing, ensure quality and minimize lot-to-lot variability.

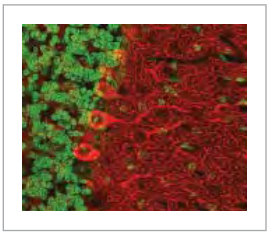
> **Cytokines**
> **Chemokines**
> **Growth Factors**
> **Receptors**
> **Proteases**
> **Adhesion Molecules**
> **Neurotrophic Factors**
> **Developmental Molecules**
> **DNA Modifying Enzymes**
> **Kinases & Phosphatases**
> **Sulfatases**
> **Inhibitors**



Features

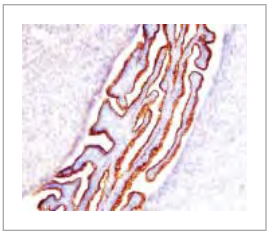
> Typically >95% purity
> Low endotoxin level
> High biological activity
> Long-term stability
> Preservative-free
> Available with BSA or carrier-free
> Available in bulk quantities
> Animal-free preparations available

All package inserts and several bioassay protocols are available on our website at **www.RnDSystems.com**



Antibodies

Choose from a vast array of affinity purified polyclonal, monoclonal, and labeled antibodies against cytokines, chemokines, receptors, adhesion molecules, proteases, inhibitors, kinases, and other related factors from a variety of species. R&D Systems antibodies are generated in several different host species and are quality-tested for exceptional performance in a broad range of applications.



Host Species

> Mouse
> Chicken
> Donkey
> Goat
> Hamster
> Rabbit
> Rat
> Sheep

Target Species

> Human
> Mouse
> Rat
> Bovine
> Canine
> Chicken
> Cotton Rat
> *Drosophila*
> Equine
> Feline
> Porcine
> Primate
> Bacterial
> Viral
> *Xenopus*
> Zebrafish

Secondary Antibodies & Conjugates

Fluorescent (Ex/Em maxima)

> NorthernLights™ - 493 (493/514)
> NorthernLights - 557 (557/574)
> NorthernLights - 637 (637/658)
> Allophycocyanin (APC) (645/660)
> Carboxyfluorescein (CFS) (492/517)
> PerCp (482, 564/675)
> Phycoerythrin (PE) (565/575)

Others

> Biotin
> Alkaline Phosphatase (AP)
> Horseradish Peroxidase (HRP)
> Cell & Tissue Staining Kits
> Agarose

ELISAs

R&D Systems offers more than 300 complete kits and development reagents for the measurement of cytokines, growth factors, adhesion molecules, eicosanoids, hormones, signal transduction molecules, and more. Complete kits are fully validated, require no development time, and are quality assured to meet the performance criteria outlined in each product manual or package insert. Development reagents contain matched antibodies and other components required for development of a working assay by the customer.

Complete Kits

Quantikine®
Colorimetric sandwich ELISAs

Quantikine HS
High sensitivity colorimetric s andwich ELISAs

Quantikine IVD®
Colorimetric sandwich ELISAs for *in vitro* diagnostic use

QuantiGlo®
Chemiluminescent ELISAs

Parameter™
Small molecule microplate-based competitive assays

Surveyor™ IC (Intracellular)
Intracellular protein ELISAs

Cell-Based ELISAs
Two color immunoenzymatic assays to permit screening of intracellular proteins using whole cells without the need to prepare cell lysates.

Development Reagents

DuoSet® ELISA Development Systems
Reagents for the development of ELISAs for cytokines, growth factors, and related molecules.

DuoSet IC (Intracellular) ELISA Development Systems
Reagents for the development of ELISAs for intracellular factors involved in apoptosis, genotoxic stress, signal transduction, and more.

Matched Antibody Pairs for ELISA Development
Design your own ELISA using our matched capture and detection antibody pairs.

Multiplex Assays/Arrays

R&D Systems has developed both antibody-spotted membrane-based arrays and antibody-coupled bead-based assays for the measurement of levels and/or phosphorylation status of multiple analytes in a single sample.

Proteome Profiler™ Arrays

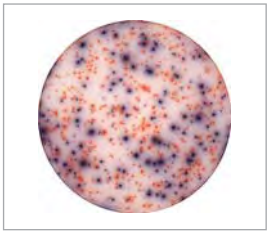
> Antibody-based arrays for cytokines, immunoreceptors, receptor tyrosine kinases, intracellular kinases, apoptosis-related molecules, angiogenesis-related molecules, and more.
> Requires no specialized equipment

Fluorokine® MAP (Multi-analyte Profiling) for the Luminex® Platform

> Validated panels of bead sets configured for cytokines, proteases, adhesion molecules, and obesity markers
> Premixed complete kits for TIMPs & TGF-βs

ELISpot & Cell-Based Assays/Reagents

R&D Systems ELISpot and cell-based assays for cytokine secretion, intracellular signaling, apoptosis, and receptor binding are ideal for observing the effects of test compounds such as growth factors, drugs, and inhibitors on specific cell populations.



ELISpot

Highly sensitive microplate-based assays to detect absolute numbers of cytokine, chemokine, or protease secreting cells.

ELISpot Development Modules

Reagents for the development of ELISpot assays for cytokines, chemokines, proteases, & more.

Dual-Color ELISpot

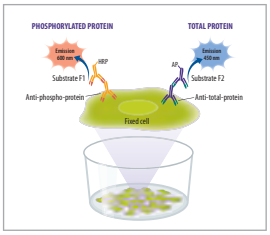
ELISpot Kits for the simultaneous detection of two analytes.

Cell-Based ELISAs

ELISAs for intracellular proteins in whole permeabilized cells - no lysates required.

Flow Cytometry Kits & Reagents

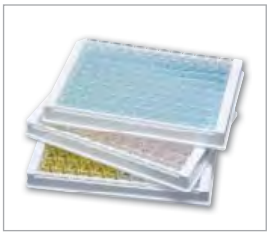
> Fluorokine® Kits (labeled cytokines used to monitor the presence of cytokine receptors)
> Antibodies for cell surface staining
> Antibodies for intracellular staining



Extracellular Matrix Reagents

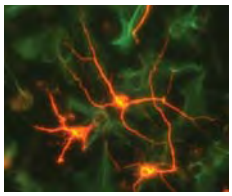
> Basement membrane extracts & cell invasion assays
> Individual extracellular matrix molecules
> Extracellular matrix-coated plates

Assay Services



Analytical Testing Service (ATS)

A sample testing service that follows applicable GLP & QSR guidelines to provide accurate and reproducible results in a timely and confidential manner.
> Clinical trial sample testing
> Biomarker testing



Stem Cell & Cell Culture/Selection

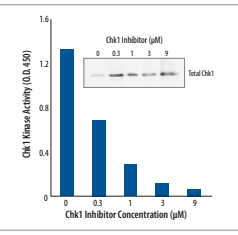
Tools for the expansion and analysis of neural, mesenchymal, hematopoietic, and embryonic stem cells are available from R&D Systems. Serum-free and serum-containing media, as well as extracellular matrix-related reagents, are also available.

Stem Cell Kits
> Expansion Kits
> Functional Identification Kits
> Differentiation Kits
> Marker Antibody & Primer Panels
> Neuronal Toxicity Assay

Primary Cells
> Rat Cortical Stem Cells
> iMEF (Irradiated Mouse Embryonic Fibroblasts)

Culture Media & Supplements
> StemXVivo™ serum-free culture media
> Methylcellulose-based reagents for colony forming cell assays
> Feeder Cell Conditioned Media

Cell Selection Kits
> Cell enrichment columns for T cell subsets & eosinophils
> MagCellect™ Kits for T cell subsets, B cells & lineage depletion
> PlusCellect™ Kits for cell enrichment & phenotyping



Activity Assays & Reagents

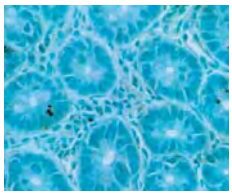
R&D Systems offers a variety of reagents and functional assays for the measurement of kinase, phosphatase, or protease activity, as well as transcription factor binding.

Kinases
> Kinase activity assays
> Active recombinant kinases

Phosphatases
> Phosphatase activity assays
> Phospho-peptide substrates

Proteases
> Protease activity assays
> Fluorogenic peptide substrates
> Active recombinant proteases

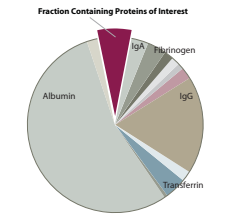
Transcription Factors
> Transcription factor binding assays



Apoptosis/Cell Viability Kits & Reagents

R&D Systems offers a variety of apoptosis-related reagents including proteins, antibodies, ELISA/activity assays, caspase inhibitors, and primer pairs. Tools are available to study Bcl-2 family members, the TNF superfamily, Caspases, Cytochrome c, IAPs, Granzymes, and more.

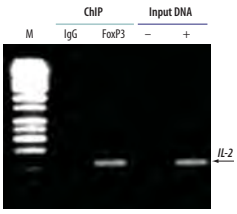
> Annexin V detection
> Caspase activity
> Cell viability/proliferation (MTT, Resazurin, XTT, Calcein)
> DNA fragmentation detection kits
> Mitochondrial membrane potential disruption
> PARP activity assay
> Proteome Profiler apoptosis array
> SOD activity assay
> TUNEL assays



Plasma/Serum Immunodepletion

R&D Systems has developed immunodepletion resins to reproducibly remove high-abundance proteins from serum or plasma. Depletion of high-abundance proteins is an essential first step for effective proteome analysis and beneficial when analyzing low-abundance proteins.

> Proteome Purify™ 12 Human Immunodepletion Resin
> Proteome Purify 2 Human Serum Protein Immunodepletion Resin
> Proteome Purify 2 Mouse Serum Protein Immunodepletion Resin



Molecular Biology Reagents

ExactaChIP™ Chromatin Immunoprecipitation Kits
R&D Systems ExactaChIP Chromatin IP Kits are designed to provide a fast and simple method for the identification of genomic DNA target sequences bound by a specific protein. Use ExactaChIP Kits to identify conditions or treatments that activate a specific transcription factor and to analyze DNA regions complexed by the transcription factor.

Primer Pairs
R&D Systems offers carefully designed and optimized primer pairs for measuring expression of specific human, mouse, and rat mRNAs by RT-PCR. All primer pair sequences are confirmed and tested to verify functionality and size.

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Ordering Information

USA & CANADIAN CUSTOMERS

Online ordering is also available for U.S. and Canadian customers via R&D Systems E-Commerce website. After finding your products, click the shopping cart symbol to create an account or to make an online purchase.

Website: www.RnDSystems.com

Phone: 1-800-343-7475

Fax: 1-612-656-4400

Mail: R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413

INTERNATIONAL DISTRIBUTORS

Please visit www.RnDSystems.com for a full list of international distributors.

FOR ALL PURCHASES, PLEASE INCLUDE THE FOLLOWING INFORMATION WITH YOUR ORDER

✓ Name & Contact Information
✓ Name of Institution
✓ Delivery Address
✓ Invoice Address
✓ Purchase Order Number* or Credit Card Number
✓ Quantity and Product Size
✓ Product Catalog Number and Description
✓ Name of Principal Investigator (optional)
✓ Telephone Number
✓ E-mail Address (optional)

For European Customers: VAT Exemption Certificate (UK) or E.C. equivalent

* New customers wishing to use a P.O. number, please call customer service at 1-800-343-7475 to set up an account.

Trademark Information

The following is a listing of trademarks used by R&D Systems, Inc. This list is subject to change at any time. Registration applications for several marks are pending and may become registered marks in the near future.

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DuoSet®
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