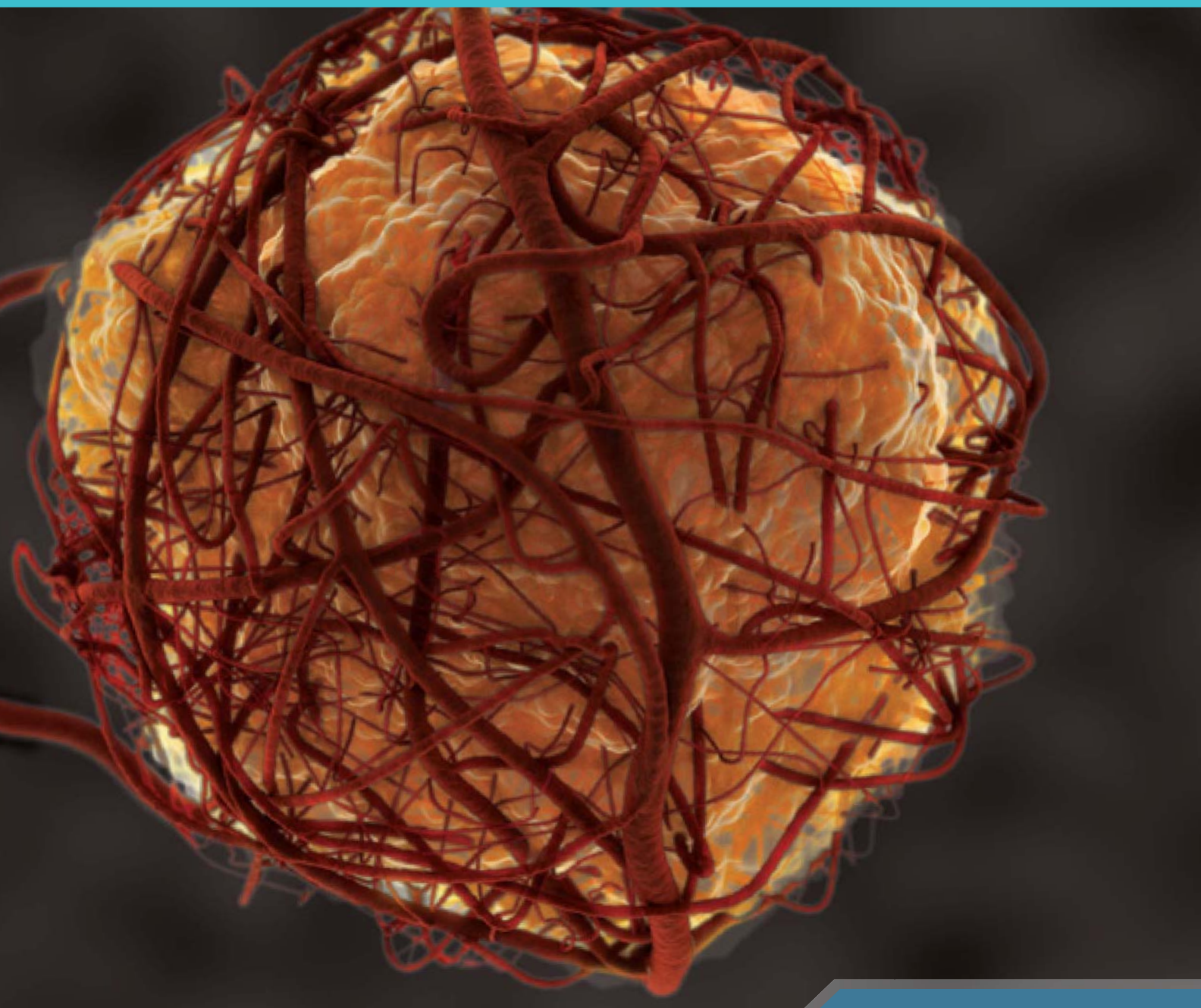
CELL SIGNALING TECHNOLOGY

Antibodies, Kits and Reagents for

Angiogenesis Research



Cell Signaling TECHNOLOGY®

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Hong, Target Validation, (left) has been with CST since 2004. **Krystyna, Clinical Assay Development**, (right) has been with CST since 2004.

Angiogenesis in Cancer

Angiogenesis is the physiological process through which new blood vessels form from larger, pre-existing blood vessels. It is required during embryogenesis and defects in or interference with this process have severe consequences for embryonic development. In adult organisms, angiogenesis plays a key role in the female reproductive system as well as normal physiological processes such as wound healing and inflammation. The process of angiogenesis can also be hijacked by tumors, which require a supply of oxygen and nutrients to support the high metabolic rate of cancer cells. Without this process, tumor growth would be suspended. Cancer cells secrete a variety of growth factors and cytokines that stimulate classical angiogenic signaling pathways and extracellular matrix remodeling. Cytokines can also induce an inflammatory response that initiates angiogenesis and consequent vascularization of the tumor (1). Sprouting angiogenesis is the first step in this neovascularization process. In response to stimuli released by tumor cells, a single endothelial cell (EC) migrates toward the angiogenic growth factors and proliferates to form sprouts of endothelial cells surrounding a lumen that is connected to the parent blood vessel (2). Tumors may also be vascularized by intussusceptive angiogenesis. This process proceeds much more quickly than sprouting and is essentially the splitting of an existing blood vessel into two new vessels (3). The ECs, pericytes and basement membrane associated with a tumor exhibit abnormalities leading to tortuous, poorly organized and leaky vasculature. However, this sub-optimal structure is often sufficient to supply the tumor with oxygen, nutrients and soluble factors that promote its survival and expansion. It is through this vascular system that some tumor cells are able to release and migrate through the circulatory system to new locations. Such metastatic colonization is a key factor in the fatal outcomes of many types of cancer, and the density of tumor angiogenesis has been linked to tumor metastasis and patient survival rates (3,4). Accordingly, angiogenesis is under investigation as an important prognostic indicator in cancer and angiogenesis inhibitors are targets in therapeutics.

References

1. Sakurai, T. and Kudo, M. (2011) *Oncology* 81 Suppl 1, 24–29.
2. Benazzi, C., Al-Dissi, A., Chau, C.H., Figg, W.D., Sarli, G. de Oliveira, J.T., and Gartner, F. (2014) *Scientific World Journal*. 2014, ePub.
3. Makanya, A.N., Hlushchuk, R. and Djonov, V.G. (2009) *Angiogenesis* 12, 113–123.
4. Jain, R.K. (2005) *Science* 307, 58–62.

A Trusted Research Partner

Cell Signaling Technology (CST) strives to be your research partner for the study of angiogenesis. As scientists we understand the importance of using antibodies that consistently work each and every time. Our highly specific antibodies directed against relevant targets in angiogenesis are painstakingly validated to work in their recommended applications, so you can have more confidence in your results. In addition, we provide siRNA, chemical modulators, control cell extracts, and kits—all validated using the same rigorous quality standards—giving you the tools you need for every step of the experimental process. Optimal antibody dilutions and recommended buffers are predetermined for you, saving you time, sample, and the trouble of additional optimization steps. Protocols and troubleshooting guides for commonly used applications are available on our website to help you get reliable results in the shortest amount of time. If you experience a problem in the lab, the same expert scientists who produced and validated your antibody will respond to your email or phone call and help you, sharing their bench experience and data from their notebooks.

Products and Tools
for Angiogenesis Research

CST offers thoroughly validated antibodies and reagents

for each stage of the experimental process

Primary Antibodies CST offers a broad range of highly specific primary antibodies to key targets in angiogenesis signaling pathways. Currently, we offer more than 200 primary antibodies against over 100 targets, including phosphorylation and other protein modification sites. Our portfolio is constantly expanding, so please check our website frequently for a complete, up-to-date product list.

Antibody Sampler Kits Antibody Sampler Kits allow for comprehensive research of multiple nodes in a pathway of interest.

ELISA Kits Scale up your analysis in a 96-well format using PathScan® ELISA Kits. (384-well plates are also available on a custom basis.)

Growth Factors and Cytokines Treat cells with growth factors or cytokines to induce or inhibit angiogenesis.

Experimental Controls Control cell extracts, control proteins, blocking peptides, and isotype controls can help verify antibody specificity- critical for accurate data analysis.

Companion Products Whatever your application, CST supports your experiments with secondary antibodies, loading controls, buffers, dyes, and other detection reagents. Our companion products work optimally with our antibodies and save time by having all the tools you need in one place. Visit **www.cellsignal.com/reagents** for a complete list of reagents by application.

We've got you covered

Our modification-specific and total protein antibody selection provides broad coverage of angiogenesis signaling events.

- ADAMTS1
- Angiopoietin-2
- CA9
- VE-Cadherin
- CBP
- Acetyl-CBP (Lys1535)
- CD31
- Cox2
- Cripto
- CYR61
- DLL4
- Endoglin
- eNOS
- Phospho-eNOS (Ser113, Thr495, Ser1177)
- EphA2
- Phospho-EphA2 (Tyr594)
- EphB1
- Phospho-Ephrin B (Tyr324/329)
- ETS-1
- Acidic FGF
- Basic FGF
- FGF Receptor 1
- Phospho-FGF Receptor (Tyr653/654)
- Phospho-FGF Receptor 1 (Tyr766)
- FGF Receptor 2
- FGF Receptor 3
- FGF Receptor 4
- FIH
- Gremlin
- HIF-1α
- Hydroxy-HIF-1α (Pro564)
- HIF-1β
- HO-1
- IGFBP2
- IGFBP3
- IGF-I Receptor
- IL-1β
- Integrin α5
- Integrin α6
- Integrin αV
- Integrin β1
- Integrin β3
- Integrin β5
- Jagged1
- Maspin
- M-CSF Receptor
- Phospho-M-CSF Receptor (Tyr546, Tyr699, Tyr708, Tyr723, Tyr809, Tyr923)
- MCP-1
- M-CSF
- Mic-1
- MMP-2
- MMP-7
- MMP-9
- NDRG1
- Phospho-NDRG1 (Ser330, Ser346)
- NDRG2
- NDRG3
- NDRG4
- Neuroplin-1
- Neuroplin-2
- Notch1
- Cleaved Notch1 (Val1744)
- Notch2
- Notch3
- Notch4
- NT5E/CD73
- PAR2
- Paxilin
- PDGF Receptor α
- Phospho-PDGF Receptor α (Tyr849)/PDGF Receptor β (Tyr849)
- Phospho-PDGF Receptor α (Tyr754, Tyr1018)
- PDGF Receptor β
- Phospho-PDGF Receptor β (Tyr740, Tyr751, Tyr771, Tyr1009, Tyr1021)
- PHD-2
- Phospholamban
- Phospho-Phospholamban (Ser16/Thr17)
- Plasminogen
- Plexin A1
- Plexin A2
- Plexin A4
- Prolactin Receptor
- RECK
- Renin
- Ron
- Semaphorin 3B
- Semaphorin 4B
- Spry1
- Syndecan 1
- Syndecan 4
- Tenascin
- Pro-TGF-α
- TGF-β
- TGF-β Receptor I
- TGF-β Receptor III
- Tie2
- Phospho-Tie2 (Tyr992, Ser1119)
- TIMP1
- TIMP2
- TIMP3
- Thymidine Phosphorylase
- uPAR
- VEGF Receptor 1
- VEGF Receptor 2
- Phospho-VEGF Receptor 2 (Tyr951, Tyr996, Tyr1175, Tyr1212)
- VEGF Receptor 3
- VHL

Visit **www.cellsignal.com/angio** to find the full range of CST antibodies, kits, modulators, and controls for these angiogenesis research targets.

Angiogenesis Modulators

Promoters of Angiogenesis

- Acidic fibroblast growth factor (aFGF)
- Angiogenin
- Basic fibroblast growth factor (bFGF)
- Epidermal growth factor (EGF)
- Granulocyte colony-stimulating factor (GM-CSF)
- Hepatocyte growth factor (HGF)
- Interleukin 8 (IL-8)
- Placental growth factor (PGF)
- Platelet derived growth factor (PDGF)
- Transforming growth factor alpha (TGF-α)
- Tumor necrosis factor alpha (TNF-α)
- Vascular endothelial growth factor (VEGF)

Inhibitors of Angiogenesis

- ADAMTS1
- Angiostatin
- Endostatin
- Interferons (alpha)
- Platelet factor 4
- Prolactin 16 kDa fragment
- Thrombospondin
- Tissue inhibitor of metalloproteinase-1 (TIMP-1)
- Tissue inhibitor of metalloproteinase-2 (TIMP-2)
- Tissue inhibitor of metalloproteinase-3 (TIMP-3)

Antibody Sampler Kits

Antibody sampler kits offer a convenient and economical means for western blot analysis of multiple nodes within a pathway of interest.

For a complete listing of our Antibody Sampler Kits: **www.cellsignal.com/abkits**

Phospho-VEGF Receptor 2 Antibody Sampler Kit #12599

Kit Includes:

Phospho-VEGF Receptor 2 (Tyr951) (15D2) Rabbit mAb #4991, Phospho-VEGF Receptor 2 (Tyr996) Antibody #2474, Phospho-VEGF Receptor 2 (Tyr1059) (D5A6) Rabbit mAb #3817, Phospho-VEGF Receptor 2 (Tyr1175) (D5B11) Rabbit mAb #3770, VEGF Receptor 2 (D5B1) Rabbit mAb #9698, Anti-rabbit IgG, HRP-linked Antibody #7074.

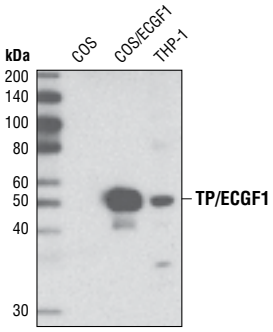
Tools to Support Your Experimental Workflow

ANGIOGENIC STIMULI

Observe expression of angiogenic stimulators or inhibitors

Increased expression of thymidine phosphorylase has been associated with solid tumors and poor prognosis.

Thymidine Phosphorylase/ECGF1 (D69B12) Rabbit mAb #4307: WB analysis of extracts from COS cells, untransfected or transfected with human ECGF1, and THP-1 cells, using #4307.

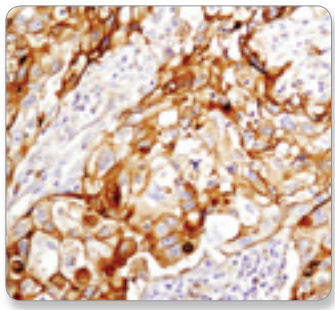


CELLULAR READOUTS

Antibodies to key cellular readout targets for angiogenesis signaling

CD73 expression on tumor cells promotes angiogenesis.

NT5E/CD73 (D7F9A) Rabbit mAb #13160: IHC analysis of paraffin-embedded human lung carcinoma using #13160.

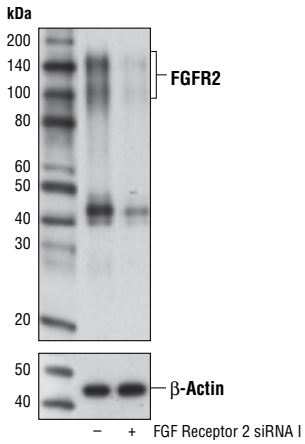


EXPERIMENTAL TOOLS

Alter protein expression with controlled knockdowns

Antibody detection can be used to confirm results of siRNA-mediated knockdown experiments.

FGF Receptor 2 (D4H9) Rabbit mAb #11835: Western blot analysis of extracts from KATO III cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® FGF Receptor 2 siRNA I (+), using FGF Receptor 2 (D4H9) Rabbit mAb #11835 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The FGF Receptor 2 (D4H9) Rabbit mAb confirms silencing of FGF Receptor 2 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

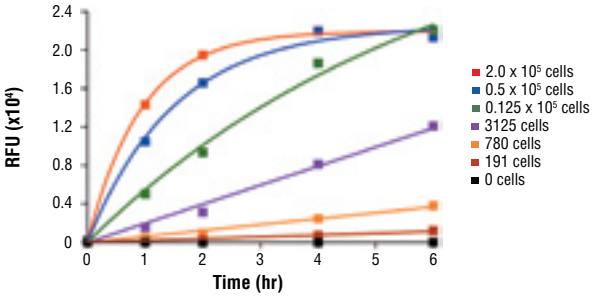


ASSAYS & KITS

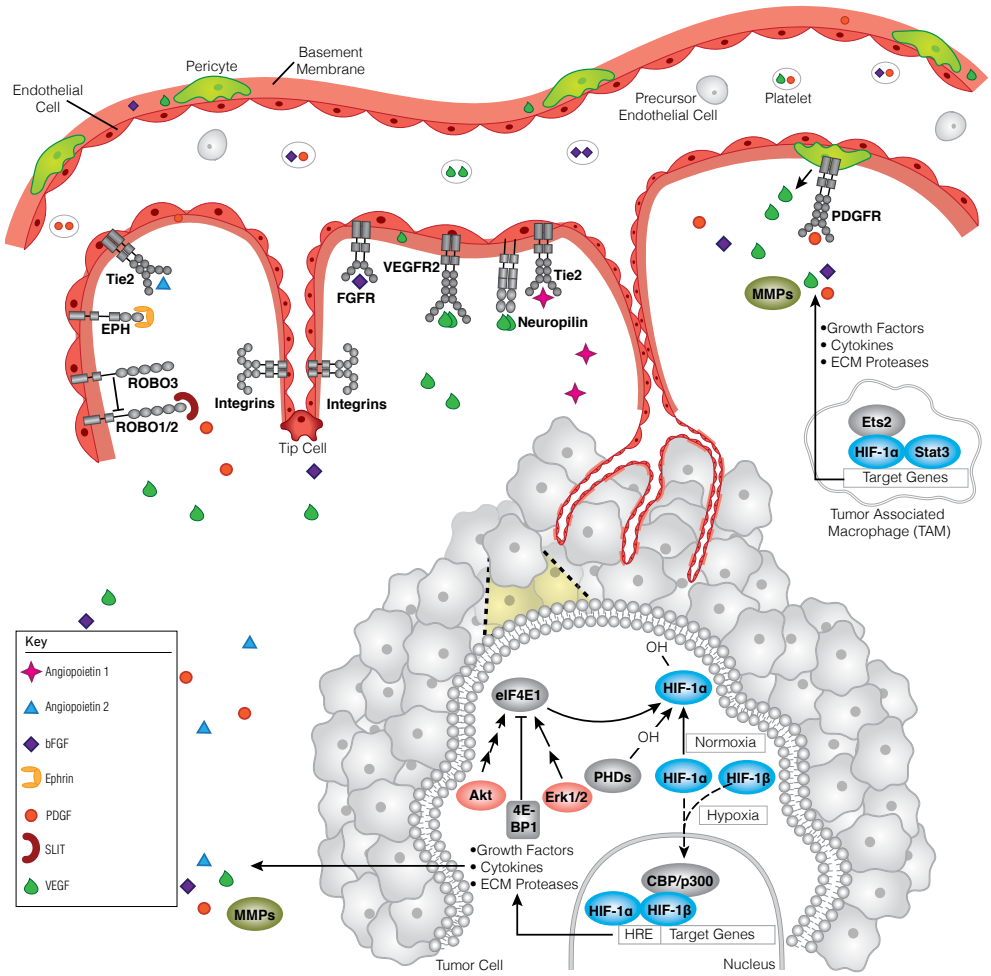
Monitor Treatment Toxicity

Measure cell viability to monitor treatment toxicity by using Reszurin to detect cellular metabolic activity.

Resazurin Cell Viability Kit #11884: HeLa cells were seeded at varying density in a 96-well plate and incubated overnight. The Resazurin solution (10% of cell culture volume) was added to the plate and relative fluorescent units were measured at 0, 1, 2, 4, and 6 hr.



Angiogenesis Signaling

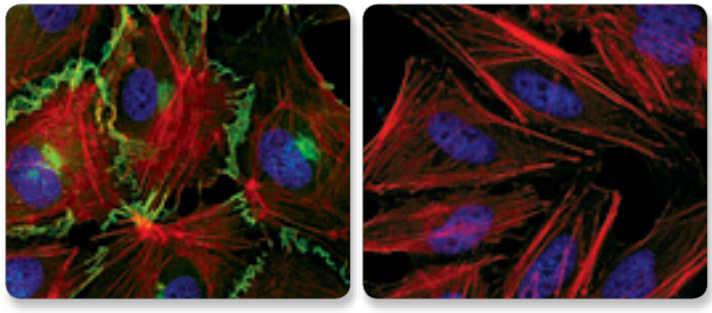


Angiogenesis Signaling in Tumor Neovascularization

Angiogenesis, the formation of new blood vessels from pre-existing blood vessels, plays a key role in tumorigenesis. When a small dormant tumor undergoes the initiation of angiogenesis, referred to as the 'angiogenic switch', it secretes factors that induce sprouting and chemotaxis of endothelial cells (ECs) towards the tumor mass. Within the hypoxic environment of the inner tumor mass the transcription factor Hypoxia-Inducible-Factor-1-alpha (HIF-1α) is stabilized and activates the expression of multiple genes contributing to the angiogenic process. HIF-1α induced proteins include Vascular Endothelial Growth Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF), which promote vascular permeability and EC growth, respectively. Other secreted factors, such as PDGF, angiopoietin 1 and angiopoietin 2 facilitate chemotaxis, while ephrins guide newly formed blood vessels through maintenance of cell-cell separation. Other HIF-1α induced gene products include matrix metalloproteinases (MMPs) that breakdown the extracellular matrix to facilitate EC migration and release associated growth factors. Certain integrins such as αVβ3 found on the surface of angiogenic ECs help the sprouting ECs adhere to the provisional Extracellular Matrix (ECM), migrate and survive. Factors secreted into the microenvironment surrounding the tumor activate tumor-associated macrophages (TAMs), that subsequently produce angiogenic factors, such as VEGF and MMPs, further promoting angiogenesis. Pericytes function as support cells enveloping the basolateral surface of ECs and regulate vasoconstriction and dilation under normal physiologic conditions. During the process of tumor angiogenesis sprouting vessels lack pericytes, which are later recruited by ECs to provide structural support that indirectly promotes tumor survival. For example, PDGF secreted by ECs acts as a ligand for PDGF receptor located on the pericyte membrane, causing pericytes to produce and secrete VEGF that signals through the endothelial VEGF receptor.

Select Reviews: Folkman, J. (2007) Nat. Rev. Drug Disc. 6, 273–286. | Guo, C., Buranych, A., Sarkar, D., Fisher, P.B., and Wang, X. (2013) Vasc. Cell. 5, 20. | Keith, B., Johnson, R.S., and Simon, M.C. (2012) Nat. Rev. Cancer 12, 9–22. | Klagsbrun, M and Eichmann, A. (2005) Cytokine and Growth Factor Rev. 16, 535–548. | Raza, A., Franklin, M.J., and Dudek, A.Z. (2010) J. Hematol. 85, 593–598. | Sakurai, T. and Kudo, M. (2011) Oncology 81 Suppl 1, 24–29. | Senger, D.R. and Davis, G.E. (2011) Cold Spring Harb. Perspect. Biol. 3, a005090. | van Hinsbergh, V.W. and Koolwijk, P. (2008) Cardiovasc. Res. 78, 203–212.

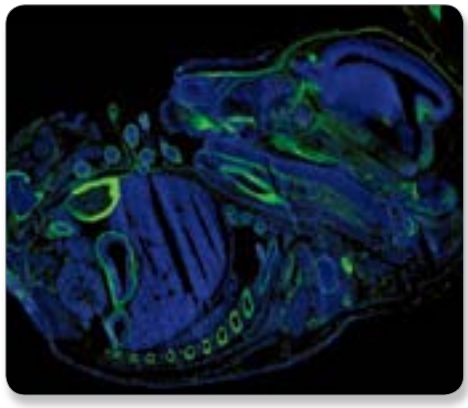
Recognize key cellular readouts for angiogenesis



Vascular permeability marker VE-Cadherin is endothelial cell specific.

VE-Cadherin (D87F2) XP® Rabbit mAb #2500: Confocal immunofluorescent analysis of HUVEC (VE-Cadherin positive; upper) and HeLa cells (VE-Cadherin negative; lower) using #2500 (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

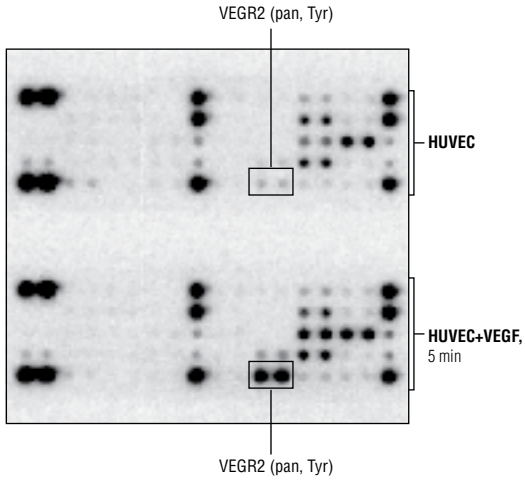
Monitor expression of receptors as angiogenic markers



Neuropilin-2 expression is inversely correlated with angiogenesis.

Neuropilin-2 (D39A5) XP® Rabbit mAb #3366: Confocal IF analysis of E14.5 mouse embryo using #3366 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

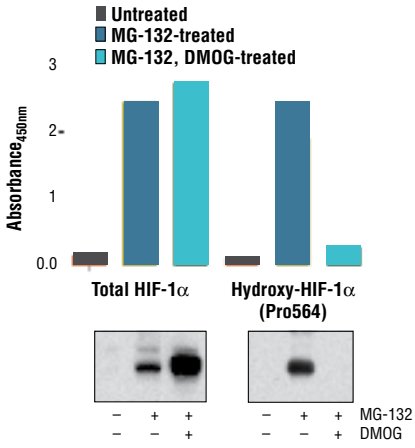
Profile responses of multiple key signaling nodes to a range of treatment conditions



The angiogenic factor VEGF stimulates VEGFR2 phosphorylation and activation.

PathScan® RTK Signaling Antibody Array Kit (Chemiluminescent Readout) #7982: HUVEC were untreated (upper panel) or treated with VEGF for 5 minutes (lower panel). Array images were captured using chemiluminescent film, with 2-5 second exposure times.

Quantify results of higher-throughput studies using PathScan® ELISA Kits



Under normoxic conditions HIF-1α is hydroxylated at Pro564, marking it for degradation.

PathScan® Total HIF-1α Sandwich ELISA Kit #13127: Treatment of HeLa cells with the hydroxylase inhibitor dimethyloxaloylglycine (DMOG) results in decreased hydroxylation of HIF-1α, as detected by the PathScan® Hydroxy-HIF-1α (Pro564) Sandwich ELISA Kit #13201, but does not affect the level of total HIF-1α detected by PathScan® Total HIF-1α Sandwich ELISA Kit #13127. Absorbance at 450 nm is shown in the top figures while corresponding western blots using a total HIF-1α antibody (left) and Hydroxy-HIF-1α (Pro564) (D43B5) XP® Rabbit mAb #3434 (right) are shown in the bottom figures. Treatment of HeLa cells with the proteasome inhibitor MG-132 #2194 stabilizes HIF-1α protein.

CST Scientists: Hongying, (left) has been with CST since 2006 and **Lori** (right) has been with CST since 2002.

Therapeutic Importance

Anti-angiogenic therapies act by preventing access to critical blood supply for tumor growth and metastasis. Cancer treatment targets include inhibitors that act on endothelial cells (ECs), block angiogenesis signaling, or prevent the degradation of extracellular matrix (1,2). Tumor xenografts or genetically manipulated mice are the most common preclinical models for assessing angiogenesis therapeutics (3).

The first FDA-approved angiogenic inhibitor was bevacizumab, a VEGF neutralizing antibody, approved for use in combination with chemotherapy or cytokine therapy for several advanced metastatic cancers and as a monotherapy for recurrent glioblastoma (3). Monoclonal antibodies targeting VEGFR2 and soluble VEGF receptors, and molecular inhibitors of VEGF receptors have successfully inhibited tumor growth in xenografts and genetically engineered mouse models (4). The FDA has also approved several tyrosine kinase inhibitors (TKIs) targeting VEGF receptors, including sorafenib, sunitinib, pazopanib, vandetanib, and axitinib (3). Recent research studies using in vitro assays and mouse models of cancer demonstrate that CD73 decreases EC contacts and promotes migration, and inhibiting CD73 expression results in reduced capillary-like structures in pulmonary microvascular ECs, reduced tumor VEGF levels and tumor angiogenesis suppression. These results point to CD73 as an intriguing therapeutic target (5,6). When combined with chemotherapy, angiogenic inhibitors have proven beneficial in reducing toxicity, whereas antiangiogenic TKIs have been less successful (7). Research continues to address challenges to antiangiogenic therapeutics, including the lack of biomarkers and the emergence of tumor resistance to angiogenesis inhibitors (8,9).

References

1. Gotink, K.J. and Verheul, H.M.K. (2010) *Angiogenesis* 13, 1–14.
2. Cook, K.M. and Figg, W.D. (2010) *CA Cancer J. Clin.* 60, 222–243.
3. Benazzi, C., Al-Dissi, A., Chau, C.H., Figg, W.D., Sarli, G. de Oliveira, J.T., and Gartner, F. (2014) *Scientific World Journal.* ePub.
4. Ebos, J.M.L. and Kerbel, R.S. (2011) *Nature Rev. Clin. Oncol.* 8, 210–221.
5. Wang, L., Tang, S., Wang, S., Xu, S., Yu, J., Zhi, X., Ou, Z., Yang, J., Zhou, P., and Shao, Z. (2013) *Clin. Exp. Metastasis* 30, 671–680.
6. Allard, B., Turcotte, M., Spring, K., Pommey, S., Royal, I., and Stagg, J. (2013) *Int. J. Cancer* 134, 1466–1473.
7. Kerbel, R.S. (2006) *Science* 312, 1171–1165.
8. Jain, R.K., Duda, D.G., Willett, C.G., Sahani, D.V., Zhu, A.X., Loeffler, J.S., Batchelor, T.T., and Sorenson, A.G. (2009) *Nature Rev. Clin. Oncol.* 6, 327–338.
9. Kerbel, R.S. (2008) *N Engl. J. Med.* 358, 2039–2049.

A trusted partner at the bench

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CST ANTIBODIES	WE DO THE RELEVANT VALIDATION, SO YOU DON'T HAVE TO...
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☑	Specific reactivity confirmed in multiple biologically relevant species and cell lines
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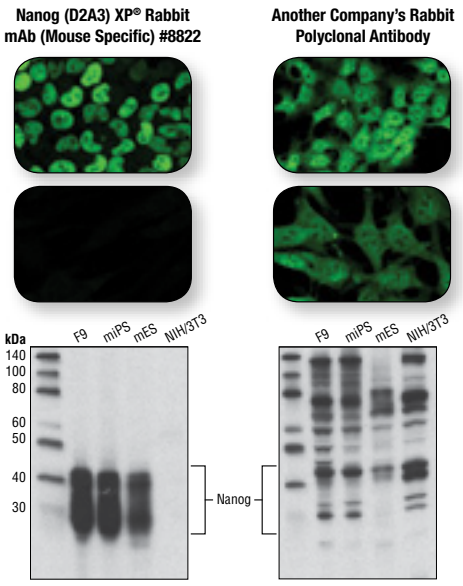
Are you confident that your antibody is specific?

WB and IF analysis show that another company's antibody lacks specificity



Seemingly comparable IF staining intensity for Nanog in F9 cells.

Non-specific IF staining in Nanog-null NIH/3T3 cells, using the antibody from another company.

In WB, The antibody from the other company recognizes multiple non-specific bands and demonstrates weak reactivity with correct bands.

Nanog (D2A3) XP® Rabbit mAb (Mouse Specific) #8822: IF analysis of F9 (Nanog-positive) or NIH/3T3 (Nanog-null) cells using #8822 or another company's antibody (upper panel). WB analysis of various cell lines using #8822 or another company's antibody (lower panels).

To learn more about CST validation visit **www.cellsignal.com/cstvalidate**

CST Technical Support

At CST, providing exceptional customer service and technical support is a top priority. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

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CST Product Scientists: Troy, (left) has been with CST since 2010 and **Christina** (right) has been with CST since 2007.

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FRONT COVER IMAGE:
Molecular rendering of tumor vascularization.

CST Scientists: Hong, (left) has been with CST since 2004, **Laurie,** (middle) has been with CST since 2009, and **Kristen,** (right) has been with CST since 2007.

FOUNDED BY RESEARCH SCIENTISTS IN 1999, Cell Signaling Technology (CST) is a private, family-owned company with over 400 employees worldwide. Active in the field of applied systems biology research, particularly as it relates to cancer, CST understands the importance of using antibodies with high levels of specificity and lot-to-lot consistency. It's why we produce all of our antibodies in house, and perform painstaking validations for multiple applications. The same CST scientists who produce our antibodies also provide technical support for customers, helping them design experiments, trouble-shoot, and achieve reliable results. We do this because that's what we'd want if we were in the lab. Because, actually, we are.

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