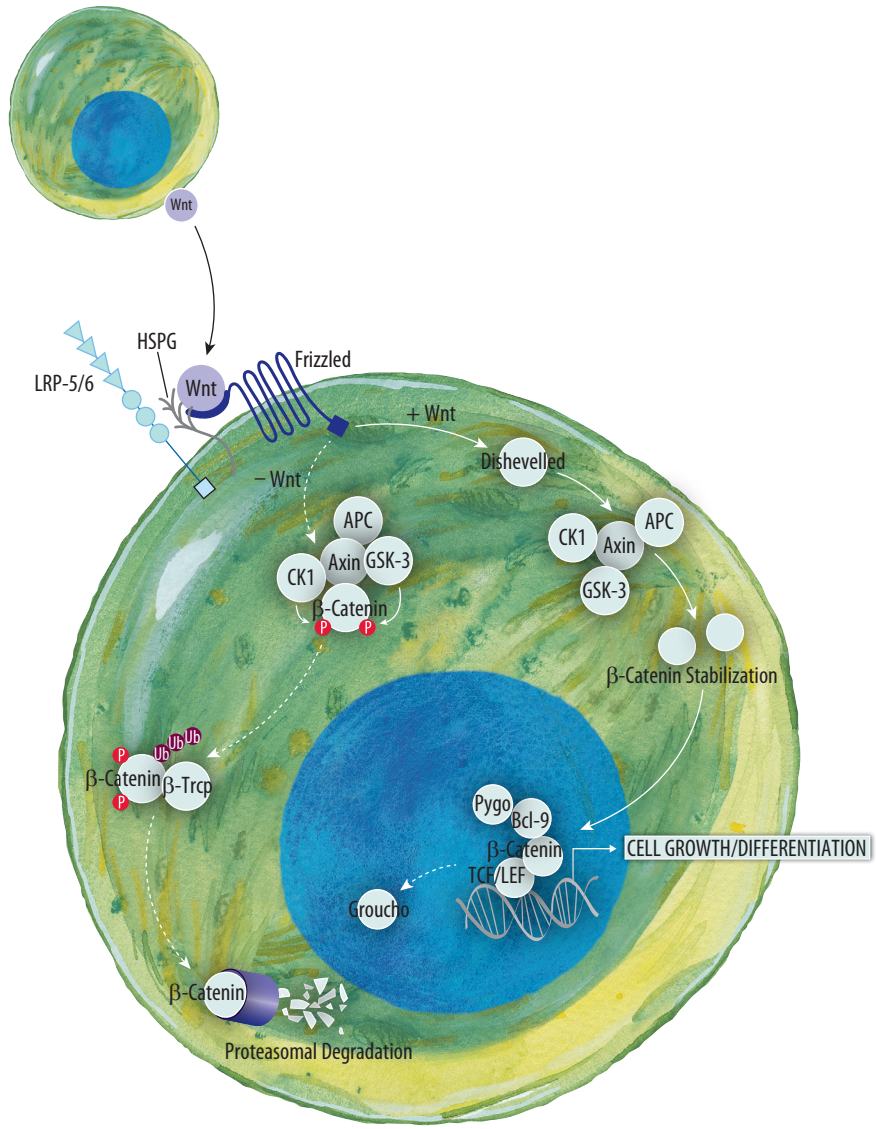
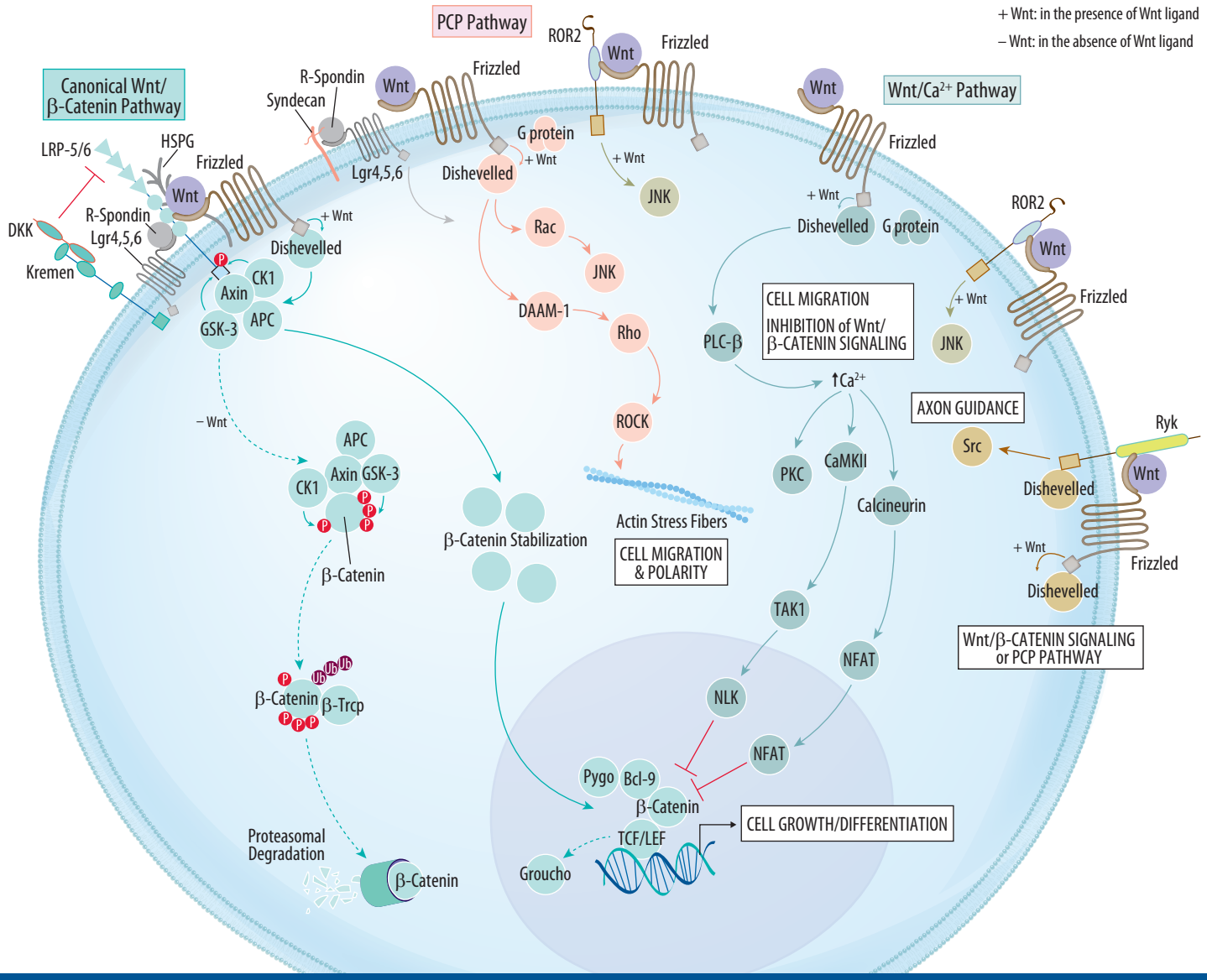
Products for Wnt Research



Wnt Signaling

Wnt signaling has a central role in embryonic development, differentiation, cell motility, cell proliferation, and adult tissue homeostasis. Wnt ligands signal through a canonical β-Catenin-dependent pathway, and at least two well-established β-Catenin-independent pathways, the planar cell polarity (PCP) pathway and the Wnt-Ca^{2+} pathway. The canonical β-Catenin-dependent pathway is initiated by association of Dishevelled with the activated Wnt receptor. Dishevelled recruits the Axin protein complex (Axin, APC, CK1, GSK-3), leading to phosphorylation of the LRP-5/6 co-receptor by Glycogen Synthase Kinase-3 (GSK-3) and Casein Kinase 1 (CK1), and inhibiting phosphorylation of β-Catenin by these kinases. Unphosphorylated β-Catenin accumulates and subsequently translocates to the nucleus, where it associates with TCF/LEF family transcription factors and co-activators, such as Bcl-9 and Pygopus (Pygo), to induce the expression of Wnt target genes. In the absence of Wnt, cytoplasmic β-Catenin is phosphorylated by CK1 and GSK-3, which creates a docking site on β-Catenin for β-Trcp, an E3 ubiquitin ligase that promotes its ubiquitination and proteasomal degradation. In addition to the β-Catenin-dependent signaling pathway, there are at least two β-Catenin-independent Wnt signaling pathways. The PCP pathway regulates cell motility and tissue polarity and involves the Rho and Rac GTPases, Rho-Kinase (ROCK), and c-Jun N-terminal kinase (JNK). R-Spondins have been shown to enhance both the canonical β-Catenin-dependent signaling pathway and the PCP pathway by binding to the leucine-rich repeat-containing, G protein-coupled receptors (Lgr), Lgr4, Lgr5, or Lgr6. R-Spondin/Lgr-enhanced Wnt/β-Catenin signaling may require ligand-receptor internalization as has also been suggested for the Wnt/Frizzled/LRP complex. The other major Wnt signaling pathway, the Wnt-Ca^{2+} pathway activates Calcium/Calmodulin-dependent Protein Kinase type II (CaMKII) and PKC. This pathway promotes cell migration, and like the PCP pathway, inhibits the β-Catenin-dependent pathway.

Wnt ligands have also been shown to signal through a number of different co-receptors, including ROR2 and Ryk, which may or may not require a Frizzled receptor. Wnt-5a/ROR2 signaling activates JNK to regulate convergent extension in *Xenopus*, inhibits the β-Catenin-dependent pathway, and may play a role in regulating the PCP pathway. In contrast, Wnt/Ryk signaling activates the β-Catenin-dependent or PCP pathways and promotes axon guidance through Src activation. Deregulation of Wnt signaling is associated with a number of developmental disorders and human diseases, including bone and renal diseases, type II diabetes, and tumorigenesis. As a result, extracellular and intracellular modulators of Wnt signaling are of great interest. R&D Systems offers a wide range of proteins, antibodies, and ELISAs for Wnt-related research.



Wnt Ligands

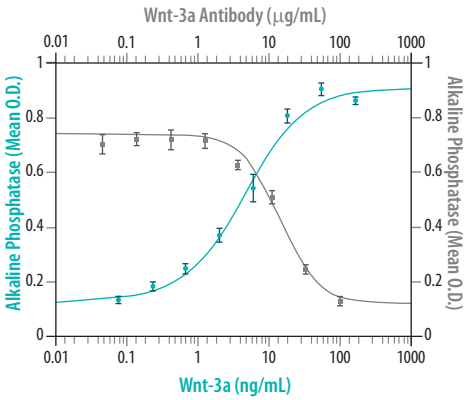
Wnt ligands are a large family of secreted glycoproteins that are cysteine-rich and highly hydrophobic. They are produced as precursor proteins that contain a short N-terminal signal sequence and a mature segment that varies in length from approximately 320-400 amino acids. In vertebrates, there are 19 different Wnt proteins whose expression is spatially and temporally regulated during development. Wnts are capable of both short and long range signaling, and their effects are mediated by binding to a receptor complex that consists of one of several Frizzled family receptors and a co-receptor, such as Lipoprotein Receptor-related Protein (LRP)-5, LRP-6, Related to tyrosine kinase (Ryk), or Receptor tyrosine kinase-like Orphan Receptor (ROR). Intracellular signaling pathways activated by Wnts regulate cell survival, cell proliferation, cell fate determination, cell polarity, tissue patterning, and tissue homeostasis.

R&D Systems Products for Wnt Ligands

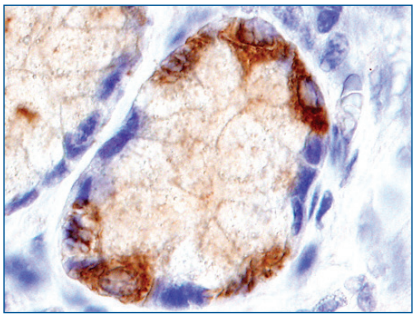
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Wnt-1		M (IHC, WB)	
Wnt-2		H (IHC, WB)	
Wnt-2b		M (IHC, WB)	
Wnt-3a	H M	H (B/N, WB) M (B/N, WB)	M
Wnt-4	H M	H (WB) M (IHC, WB)	
Wnt-5a	H M	H (IHC, WB) M (IHC, WB) R (IHC, WB)	
Wnt-5b	M	M (IHC)	
Wnt-6		H (IHC, WB)	
Wnt-7a	H	H (IHC, WB)	

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Wnt-7b		H (IHC, WB)	
Wnt-8a		M (IHC, WB)	
Wnt-8b		H (IHC, WB) M (IHC, WB)	
Wnt-9a		H (IHC, WB)	
Wnt-9b	M	H (WB) M (IHC, WB)	
Wnt-10a		H (WB) M (WB)	
Wnt-10b	H M	M (WB)	
Wnt-11	H	H (IHC, WB) M (IHC, WB)	

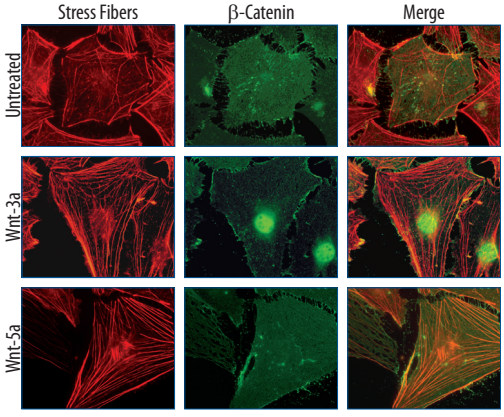
Species Key: H Human M Mouse R Rat Application Key: **B/N** Blocking/Neutralization **IHC** Immunohistochemistry **WB** Western blot



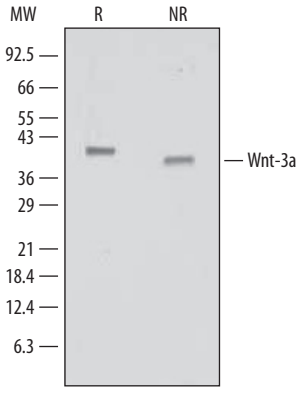
Wnt-3a-induced Alkaline Phosphatase Production and Neutralization. The MC3T3-E1 mouse preosteoblast cell line was treated with increasing concentrations of Recombinant Human Wnt-3a (Catalog # 5036-WN) and alkaline phosphatase production was assessed (aqua line). The effect induced by 20 ng/mL Recombinant Human Wnt-3a was neutralized in a dose-dependent manner using a Rat Anti-Human/Mouse Wnt-3a Monoclonal Antibody (Catalog # MAB1324; gray line).



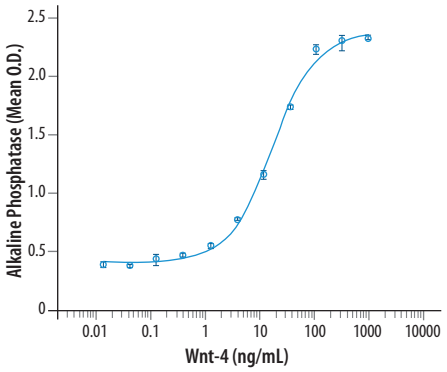
Detection of Wnt-2 in Human Stomach Cancer. Wnt-2 was detected in a paraffin-embedded human stomach cancer tissue section using a Goat Anti-Human Wnt-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3464). The section was subjected to antigen retrieval using the Basic Antigen Retrieval Kit (Catalog # CTS013) and stained using the Anti-Goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown). The tissue was counterstained with hematoxylin (blue).



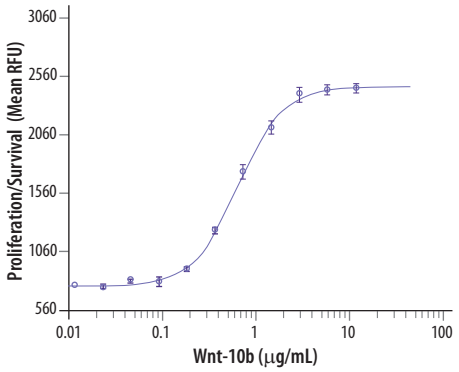
Detection of Wnt-3a and Wnt-5a Bioactivity in Mouse Fibroblasts. Recombinant Mouse Wnt-3a (Catalog # 1324-WN) and Recombinant Human/Mouse Wnt-5a (Catalog # 645-WN) promote stress fiber formation in NIH-3T3 mouse embryonic fibroblast cells, while only Wnt-3a promotes nuclear β-Catenin accumulation. *Images courtesy of Dr. Raymond Habas, Robert Wood Johnson School of Medicine, Piscataway, NJ.*



The Purity of Recombinant Human Wnt-3a. Recombinant Human Wnt-3a (Catalog # 5036-WN), 1 µg/lane, was loaded on a 4-20% SDS-PAGE gel under reducing (R) and non-reducing (NR) conditions. The gel was silver-stained. MW=Molecular weight markers.



Wnt-4 Induces Alkaline Phosphatase Production. The MC3T3-E1 mouse preosteoblast cell line was treated with increasing concentrations of Recombinant Human Wnt-4 (Catalog # 6076-WN). Alkaline phosphatase production was assessed.



Wnt-10b Promotes Intestinal Epithelial Cell Proliferation. The IEC-18 rat small intestinal epithelial cell line was grown in the presence of increasing concentrations of Recombinant Mouse Wnt-10b (Catalog # 2110-WN). Cell proliferation was assessed in a fluorometric assay using the redox sensitive dye, Resazurin (Catalog # AR002).

Wnt Receptors

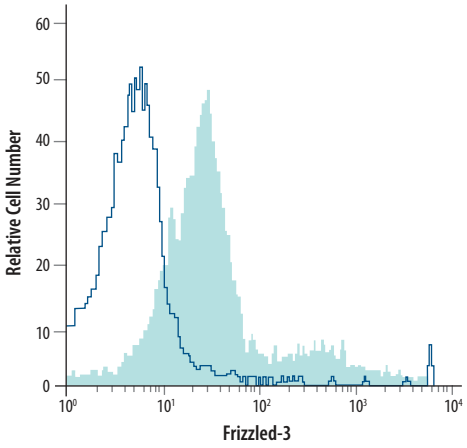
Frizzled receptors are seven transmembrane G protein-coupled receptors for the Wnt family of glycoproteins. This receptor family contains at least ten different members that mediate distinct, tissue-specific effects. Structurally, all members of the Frizzled family are similar. Each contains a divergent N-terminal signal peptide, a highly conserved extracellular cysteine-rich domain (CRD), a variable length linker region, a seven-pass transmembrane domain, and a variable length C-terminal end. Wnt ligands bind to Frizzled receptors through the CRD, and may require a co-receptor, such as LRP-5, LRP-6, ROR, or Ryk, to activate either the canonical Wnt/β-Catenin signaling pathway or one of the β-Catenin-independent, non-canonical signaling pathways. Since Wnt ligands have different affinities for different Frizzled receptors, activation of a given Wnt/Frizzled signaling cascade is dependent on the Wnt ligand and the cellular context of the interaction.

R&D Systems Products for Wnt Receptors

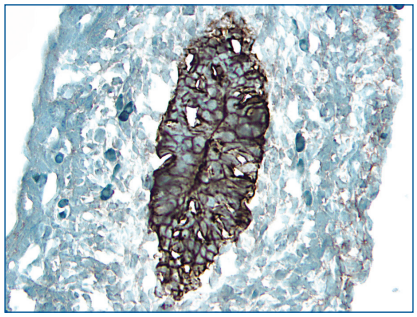
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Cripto	H M	H (B/N, E, FC, WB) M (B/N, FC, IHC, WB)	H
Frizzled-1	H M	H (FC, IHC, WB) M (FC, IHC, WB)	
Frizzled-2	M	M (WB)	
Frizzled-3		H (FC, IHC, WB) M (FC, IHC, WB)	
Frizzled-4	H M	H (FC, IHC, WB) M (FC, IHC, WB)	
Frizzled-5	H	H (WB)	
Frizzled-6		H (FC, WB) M (FC, IHC, WB)	
Frizzled-7	H M	H (FC, IHC) M (FC, IHC, WB)	
Frizzled-8	H M	M (IHC, WB)	
Frizzled-9		M (IHC, WB)	
Frizzled-10	H		
LRP-1		H (FC, WB)	
LRP-1 Cluster II	H	H (WB)	
LRP-1 Cluster III	H	H (IHC)	

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
LRP-1 Cluster IV	H		
LRP-1B		H (WB)	
LRP-4		H (IHC, WB) R (WB)	
LRP-5		H (WB)	
LRP-6	H M	H (FC, IHC, WB) M (WB)	
MuSK		H (WB) R (B/N, WB)	
PTK7		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
ROR1		H (FC, WB)	H
ROR2		H (FC, WB)	H
Ryk		H (WB) M (IHC, WB)	
Vang-like Protein 1/ VANGL1		H (IHC, WB)	
Vang-like Protein 2/ VANGL2		H (IHC, WB) M (IHC, WB) R (IHC, WB)	

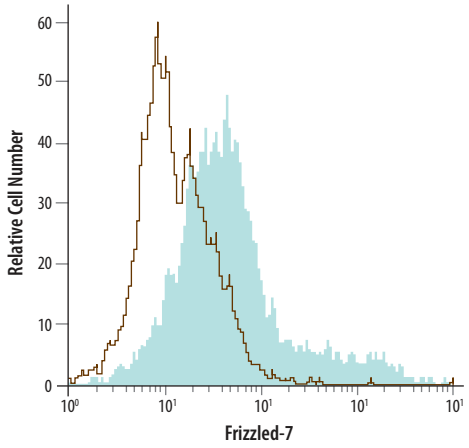
Species Key: H Human M Mouse R Rat Application Key: **FC** Flow Cytometry **IHC** Immunohistochemistry **WB** Western blot



Detection of Frizzled-3 by Flow Cytometry. The HEK293 human embryonic kidney cell line was treated with retinoic acid and then stained with an APC-conjugated Rat Anti-Human/Mouse Frizzled-3 Monoclonal Antibody (Catalog # FAB1001A; filled histogram) or an APC-conjugated Rat IgG_{2A} Isotype Control (Catalog # IC006A; open histogram).



Detection of Frizzled-1 in Mouse Intestine. Frizzled-1 was detected in a cryostat tissue section of embryonic mouse intestine (E13) using a Goat Anti-Mouse Frizzled-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1120). The tissue was stained using the Anti-Goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).



Detection of Frizzled-7 by Flow Cytometry. The HEK293 human embryonic kidney cell line was stained with a PE-conjugated Rat Anti-Human/Mouse Frizzled-7 Monoclonal Antibody (Catalog # FAB1981P; filled histogram) or a PE-conjugated Rat IgG_{2A} Isotype Control (Catalog # IC006P; open histogram).

Wnt Inhibitors

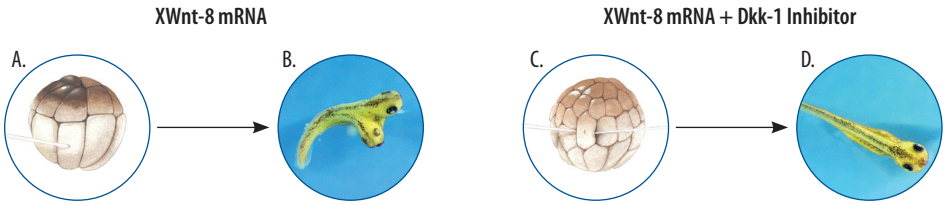
Wnt signaling can be inhibited by several known antagonists that bind either to the Wnt ligand itself, or to Wnt receptors. Proteins that act as Wnt antagonists include Dickkopf (Dkk) proteins, Wnt Inhibitory Factor-1 (WIF-1), and secreted Frizzled-Related Proteins (sFRPs). The Dkk family of secreted proteins includes Dkk-1-4 and the Dkk-3-homologous protein, Soggy. Dkk proteins function as antagonists of canonical Wnt signaling by preventing the LRP-5/6 co-receptor from interacting with Wnt-Frizzled complexes, or by binding to Kremen-1 or -2 and triggering the internalization of LRP-5/6. In contrast, both WIF-1 and sFRPs antagonize Wnt signaling by binding directly to Wnt proteins and inhibiting their activity. WIF-1 is a secreted protein that can bind to Wnts through its WIF domain. Members of the sFRP family (sFRP-1-5) bind to Wnt proteins through their N-terminal cysteine-rich domains (CRD), which are homologous to the CRDs found in the Frizzled family of Wnt receptors. Although sFRPs are generally thought to function as Wnt antagonists, they may also enhance Wnt signaling by stabilizing or transporting Wnt ligands. In addition to binding to Wnt proteins, sFRPs can also inhibit Wnt signaling pathways by binding directly to Frizzled receptors. Reduced sFRP expression is associated with aberrant activation of Wnt signaling and tumorigenesis.

R&D Systems Products for Wnt Inhibitors

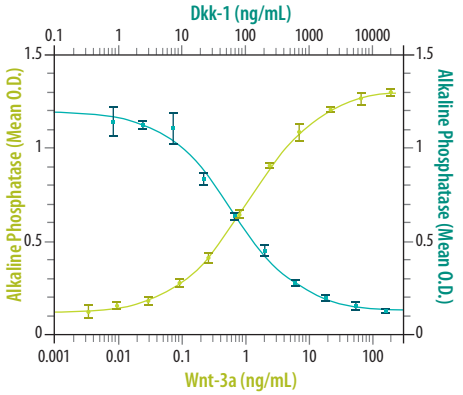
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Dkk-1	H M R	H (B/N, E, IHC, WB) M (E, IHC, WB) R (WB)	H M
Dkk-2	H M	M (IHC, WB)	
Dkk-3	H	H (E, IHC, WB) M (WB)	H
Dkk-4	H M	H (E, WB) M (WB)	H
Draxin/Neucrin	H M	H (IHC, WB) M (IHC, WB) R (IHC, WB)	
sFRP-1	H	H (WB)	
sFRP-2	M	M (WB)	
sFRP-3	H M	H (E, WB) M (E, IHC, WB)	H

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
sFRP-4	H	H (WB)	
sFRP-5	H M		
IGFBP-4	H	H (B/N, E, IHC, WB)	H
Sclerostin/SOST	H M	H (E, WB) M (IHC, WB)	H
Soggy-1/DkkL1	H M	H (WB) M (WB)	M
USAG1/WISE	H	H (WB)	
WIF-1	H	H (E, IHC, WB) M (WB)	H

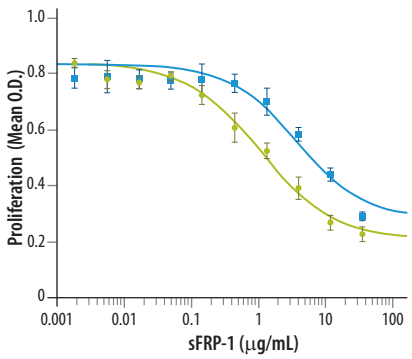
Species Key: H Human M Mouse R Rat Application Key: **B/N** Blocking/Neutralization **E** ELISA Capture and/or Detection **IHC** Immunohistochemistry **WB** Western blot



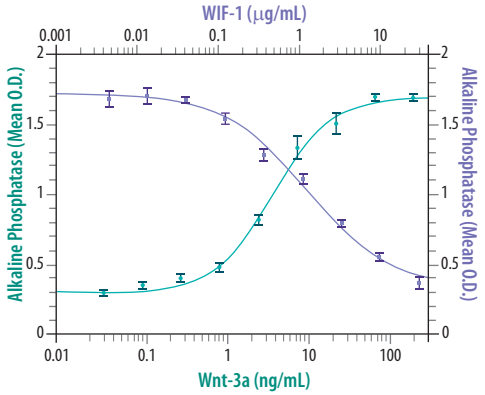
Dkk-1 Antagonizes Wnt Activity during *Xenopus* Development. A. *Xenopus* Wnt-8 mRNA was injected into a ventral/vegetal blastomere of a 16-cell stage *Xenopus* embryo. **B.** Injection of XWnt-8 mRNA leads to the formation of a secondary axis in a representative *Xenopus* embryo (stage 35). **C.** To counter the effects of XWnt-8 mRNA, Recombinant Human Dkk-1 (Catalog # 5439-DK), a Wnt antagonist, was injected into the spaces near the XWnt-8 mRNA injection site at the 64-cell stage. **D.** Injection of Recombinant Human Dkk-1 following injection of XWnt-8 mRNA prevents the development of a secondary axis.



Dkk-1 Inhibits Wnt-3a-induced Alkaline Phosphatase Production. The MC3T3-E1 mouse preosteoblast cell line was treated with increasing concentrations of Recombinant Mouse Wnt-3a (Catalog # 1324-WN) and alkaline phosphatase production was assessed (light green line). The effect induced by 10 ng/mL Recombinant Mouse Wnt-3a was inhibited by treating the cells with increasing concentrations of Recombinant Human Dkk-1 (Catalog # 5439-DK; dark green line).



sFRP-1 Inhibits Cell Growth. The HeLa human cervical epithelial carcinoma cell line was treated with Histidine-tagged Recombinant Human sFRP-1 (Catalog # 1384-SF; blue line) or Recombinant Human sFRP-1 without the histidine tag (Catalog # 5396-SF; green line). Cell proliferation was determined using the MTT Cell Proliferation/ Viability Assay (Catalog # 4890-025-K).



WIF-1 Inhibits Wnt-3a-induced Alkaline Phosphatase Production. The MC3T3-E1 mouse preosteoblast cell line was treated with increasing concentrations of Recombinant Mouse Wnt-3a (Catalog # 1324-WN) and alkaline phosphatase production was assessed (dark green line). The effect induced by 10 ng/mL Recombinant Mouse Wnt-3a was inhibited by treating the cells with increasing concentrations of Recombinant Human WIF-1 (Catalog # 1341-WF; purple line).

Wnt Modulators

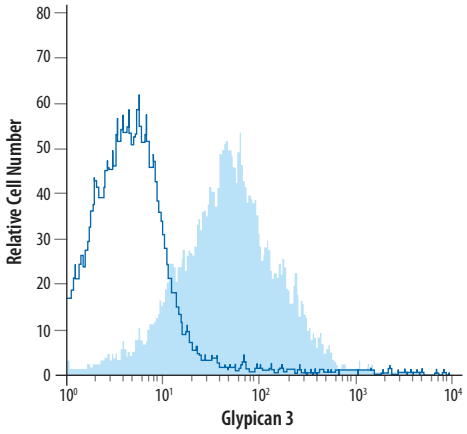
A number of proteins can either enhance or inhibit Wnt signaling, including Glypicans, R-Spondin proteins, Kremen-1, Kremen-2, and Norrin. Glypicans are a family of six heparan sulfate proteoglycans that are anchored to the plasma membrane by a glycosylphosphatidylinositol linkage. These proteins enhance Wnt signaling by stabilizing the interaction between Wnt proteins and Frizzled receptors. Members of the R-Spondin family of proteins (Rspo1-4) also function as positive regulators of Wnt/β-Catenin signaling by interfering with Dkk-1-mediated internalization of the Wnt co-receptor, LRP-6. In contrast, Kremen-1 and Kremen-2 antagonize Wnt signaling by forming a complex with Dkk proteins and LRP-5/6 that stimulates LRP-5/6 internalization. Like the R-Spondin and Kremen proteins, MESDC2 and Sclerostin (SOST) also regulate Wnt signaling through LRP-5/6. MESDC2 is required for proper folding and expression of LRP-5/6, while SOST binds LRP-5/6 and inhibits its ability to function as a co-receptor. Other proteins may regulate Wnt signaling by directly binding to Wnt ligands or Wnt receptors. For example, Norrin is a secreted protein associated with the extracellular matrix that is not related to the Wnt family, but binds to Frizzled-4/LRP and activates the canonical Wnt signaling pathway.

R&D Systems Products for Wnt Modulators

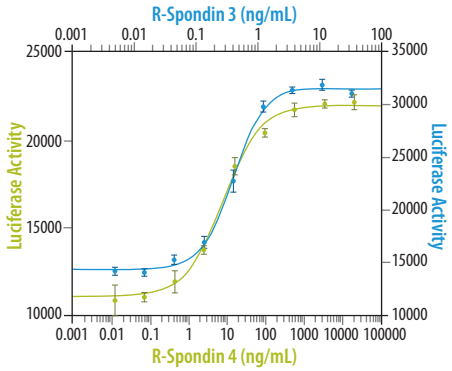
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Biglycan	H	H (IHC, WB)	
CTHRC1		M (IHC, WB)	
Glypican 1	H M	H (FC, IHC, WB)	
Glypican 3	H M	H (FC, IHC, WB)	
Glypican 5	H M	H (FC, WB) M (FC, WB)	
Kremen-1	M	H (IHC, WB) M (E, FC, IHC, WB)	M
Kremen-2	H M R	H (FC, WB) M (WB)	
MESDC2	M	H (IHC, WB) M (IHC, WB)	
MFRP	H	H (IHC, WB) M (IHC, WB)	
Myocilin		H (WB)	
NeuroD1		H (IHC, WB) M (IHC, WB)	
Norrin	H M	H (B/N, IHC, WB) M (WB)	

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Nucleoredoxin		H (WB)	
Sclerostin/SOST	H M	H (E, WB) M (IHC, WB)	H
R-Spondin 1	H M	H (B/N, WB) M (WB)	
R-Spondin 2	H M	H (WB)	
R-Spondin 3	H M	H (B/N, WB) M (WB)	
R-Spondin 4	H M	M (WB)	
Shisa-4		H (WB) M (WB)	
Syndecan-1	H M	H (E, FC, IHC, IP, WB) M (FC, IHC, IP, WB)	H
Syndecan-2	H	H (FC, IHC) M (FC, IHC, WB)	
Syndecan-3	H M	H (FC, IHC, WB) M (IHC, WB)	
Syndecan-4	H M	H (FC, IHC, WB)	

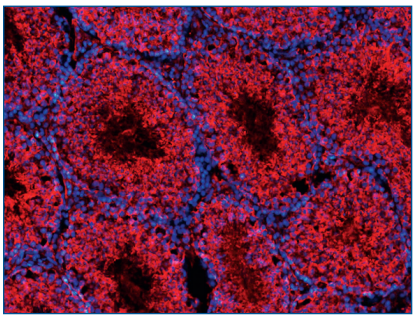
Species Key: H Human M Mouse R Rat Application Key: **B/N** Blocking/Neutralization **E** ELISA Capture and/or Detection **FC** Flow Cytometry **IHC** Immunohistochemistry **IP** Immunoprecipitation **WB** Western blot



Detection of Glypican 3 by Flow Cytometry. The HepG2 human hepatocellular carcinoma cell line was stained with an APC-conjugated Mouse Anti-Human Glypican 3 Monoclonal Antibody (Catalog # FAB2119A; filled histogram) or an APC-conjugated Mouse IgG_{2A} Isotype Control (Catalog # IC003A; open histogram).



R-Spondin 3 and R-Spondin 4 Induce β-Catenin-responsive Transcriptional Activation. In the presence of 5 ng/mL (blue line) or 10 ng/mL (green line) Recombinant Mouse Wnt-3a (Catalog # 1324-WN), increasing concentrations of Recombinant Mouse R-Spondin 3 (Catalog # 4120-RS; blue line) and Recombinant Mouse R-Spondin 4 (Catalog # 4106-RS; green line) stimulate transcriptional activation of the TOPflash β-Catenin/TCF reporter in the HEK293T human kidney cell line.



Detection of MESDC2 in Mouse Testis. Mesoderm development candidate 2 (MESDC2) was detected in perfusion-fixed, frozen sections of mouse testis using a Goat Anti-Mouse MESDC2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4545). The tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001; red) and nuclei were counterstained with DAPI (blue).

Wnt Intracellular Signaling

Multiple proteins are involved in mediating Wnt signaling. Proteins that promote the canonical β-Catenin-dependent signaling pathway include GSK-3, CK1, Dishevelled, β-Catenin, and co-activators such as Bcl-9 and Pygopus. Negative regulators of this pathway include APC, Axin-1, GSK-3, and CK1. In the absence of Wnt, GSK-3 and CK1 in the Axin-1 complex constitutively phosphorylate β-Catenin, targeting it for ubiquitin-mediated proteasomal degradation. In the presence of Wnt, the Axin protein complex is recruited to the activated Wnt receptor and GSK-3 and CK1 phosphorylate LRP-5/6. Translocation of the Axin-1 complex disrupts β-Catenin phosphorylation and degradation, allowing β-Catenin to accumulate in the nucleus and regulate the expression of specific target genes that promote cell growth and differentiation. Intracellular proteins involved in the Wnt/PCP signaling pathway regulate cell migration and tissue polarity. These proteins include the Rho and Rac GTPases, JNK, and ROCK. Another group of intracellular proteins regulates cell migration through the Wnt/Ca^{2+} pathway. Proteins required for this signaling pathway include Phospholipase C-beta (PLC-β), PKC, CaMKII, Calcineurin, Nemo-Like Kinase (NLK), and Nuclear Factor of Activated T Cells (NFAT).

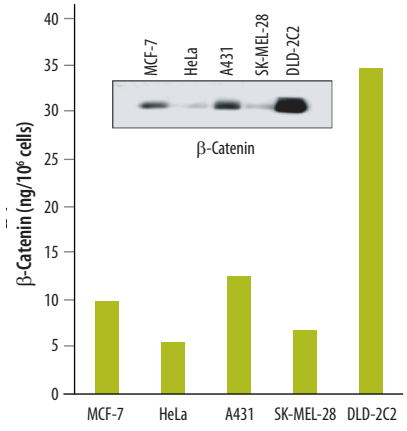
R&D Systems Products for Wnt Intracellular Signaling Molecules

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
c-Abl		H (WB)	
APC		H (IHC, WB)	
ASCL1/Mash1		M (IHC, WB)	
ASCL2/Mash2		H (IHC, WB)	
Axin-1		H (WB) M (WB) R (WB)	
Axin-2		H (IHC)	
β-Catenin		H (ChIP, FC, IHC, WB) M (ChIP, FC, IHC, WB) R (ChIP, FC, IHC, WB)	H
Bcl-9		H (WB)	
Bcl9-2		H (WB)	
Calcineurin	H		
Calcineurin A		H (WB) M (WB) R (WB)	
Calcineurin B		H (WB) M (WB) R (WB)	
CaM Kinase II		H (WB) M (WB) R (WB) B (WB) Ch (WB) X (WB)	
CaM Kinase IIα		H (IHC)	
CaM Kinase IIδ		H (IHC, WB)	
Casein Kinase 1α/CK1α		H (WB) M (WB) R (WB)	
Casein Kinase 1γ/CK1γ		H (WB) M (WB) R (WB)	
Casein Kinase 1δ/CK1δ		H (WB) M (WB) R (WB)	
Casein Kinase 1ε/CK1ε		H (WB) M (WB) R (WB)	
Casein Kinase 2β/CK2β		H (WB) M (WB) R (WB)	
Ccd1/DIXDC1		M (IHC, WB)	
CREB		H (ChIP, IHC, WB) M (WB) R (WB)	H M R
DISC1		H (IHC, WB)	

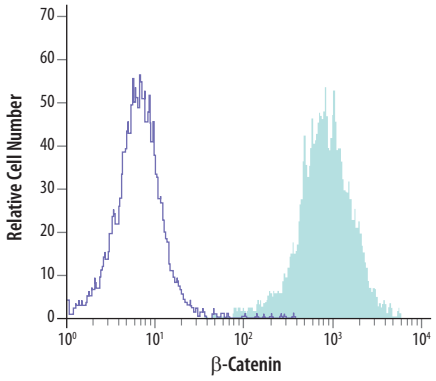
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Dishevelled-1		H (IHC, WB)	
Dishevelled-2		H (WB)	
Dishevelled-3		H (IHC, WB)	
c-Fos		H (WB)	
GSK-3α/β		H (FC, IHC, WB) M (FC, IHC, WB) R (FC, IHC, WB)	H M R
GSK-3α		H (IHC, WB) M (IHC, WB) R (IHC, WB)	H
GSK-3β	H	H (FC, IHC, WB) M (FC, WB) R (FC, WB)	H M R
HIC5/TGFB1I1		H (WB)	
ICAT		H (WB)	
JNK		H (IHC, WB) M (IHC, WB) R (IHC, WB)	H M R
JNK1	M	H (IHC, WB) M (IHC, WB) R (IHC, WB)	
JNK1/JNK2		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
JNK2		H (IHC, WB) M (IHC, WB) R (IHC, WB)	H M R
JunB		H (WB)	
c-Jun		H (IHC, WB) M (IHC, WB)	
JunD		H (WB) M (WB)	
MKK7		H (IHC, WB)	
Neuro D1		H (IHC, WB) M (IHC, WB)	
Nucleoredoxin		H (WB)	
PKCα		H (WB) M (WB) R (WB)	
PKCβ1		H (IHC, WB) R (IHC, WB)	
PKCβ2		H (WB) M (WB)	

Species Key: H Human M Mouse R Rat B Bovine Ch Chicken X *Xenopus* Application Key: **ChIP** Chromatin Immunoprecipitation **FC** Flow Cytometry **IHC** Immunohistochemistry **WB** Western blot

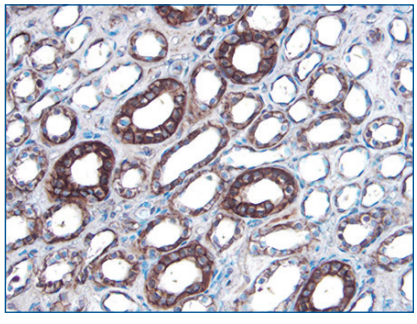
For Ubiquitin Research products, please visit:
www.RnDSystems.com/Ubiquitin



Detection of Intracellular β-Catenin using the DuoSet® IC ELISA Development System. The levels of β-Catenin in lysates prepared from the MCF-7 human breast adenocarcinoma, HeLa human cervical epithelial carcinoma, A431 human epithelial carcinoma, SK-MEL-28 human malignant melanoma, and DLD-2C2 human colorectal adrenocarcinoma cell lines were assessed using the Human Total β-Catenin DuoSet IC ELISA (Catalog # DYC1329; bar graph). The results of the DuoSet IC ELISA are consistent with the relative levels of β-Catenin detected by Western blot analysis (inset).



Intracellular Detection of β-Catenin by Flow Cytometry. The HeLa human cervical epithelial carcinoma cell line was intracellularly stained with an APC-conjugated Mouse Anti-Human β-Catenin Monoclonal Antibody (Catalog # IC13292A; filled histogram) or an APC-conjugated Mouse IgG_{2A} Isotype Control (Catalog # IC003A; open histogram).



Detection of β-Catenin in Human Kidney Cancer. β-Catenin was detected in immersion-fixed, paraffin-embedded human kidney cancer sections using a Goat Anti-Human/Mouse/Rat β-Catenin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1329). The tissue was stained using the Anti-Goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
TEL: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
www.RnDSystems.com

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

Change Service Requested

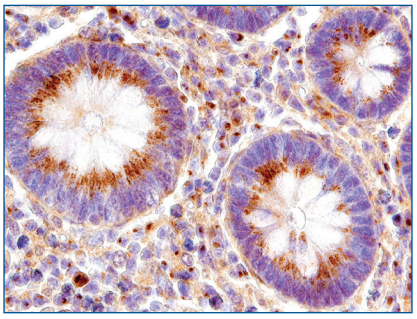
Wnt Intracellular Signaling, continued

R&D Systems Products for Wnt Intracellular Signaling Molecules

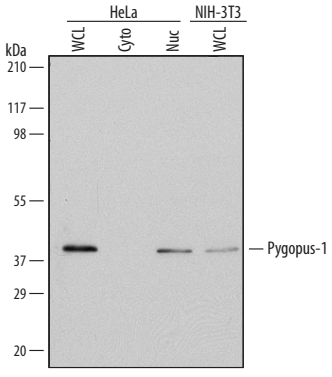
MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
PKCγ		H (WB) M (WB) R (WB)	
PKCδ	H	H (WB)	
PKCε		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
PKCζ		H (WB)	
PKCθ		H (FC, WB) M (WB)	
PKCι/λ		H (WB) M (WB)	
PKCι/λ/ζ		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
PKCν		H (WB)	
Pygopus-1		H (WB) M (WB)	

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
Pygopus-2		H (IHC, WB)	
ROCK1	H	H (WB) M (WB) R (WB)	
ROCK2		H (WB) M (WB) R (WB)	
TAK1		H (WB)	
TCF-2/HNF-1B		H (IHC, WB)	
TCF-3/E2A		H (IHC, WB)	
TCF-7/TCF-1		H (IHC, WB)	
TCF7L1/TCF3		H (IHC, WB) M (WB)	
TCF-12/HTF-4		H (IHC, WB)	

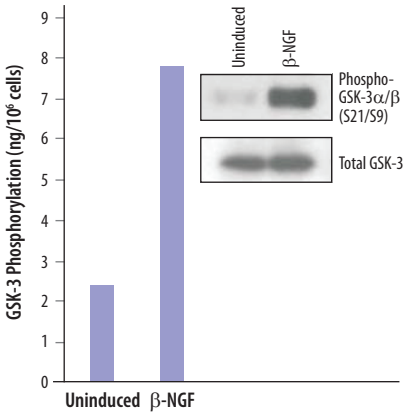
Species Key: H Human M Mouse R Rat B Bovine Ch Chicken X *Xenopus* Application Key: **ChIP** Chromatin Immunoprecipitation **FC** Flow Cytometry **IHC** Immunohistochemistry **WB** Western blot



Detection of ASCL2/Mash2 in Human Intestine. Achaete scute complex-like 2 (ASCL2/Mash2) was detected in immersion-fixed, paraffin-embedded sections of human intestine using a Sheep Anti-Human ASCL2/Mash2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6539). The tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS019; brown) and counterstained with hematoxylin (blue).



Detection of Human and Mouse Pygopus-1 by Western Blot. Whole cell lysate (WCL; 30 µg), cytoplasmic extract (Cyto; 20 µg), and nuclear extract (Nuc; 10 µg) prepared from the HeLa human cervical epithelial carcinoma cell line, and WCL prepared from the NIH-3T3 mouse embryonic fibroblast cell line were immunoblotted using a Mouse Anti-Human/Mouse Pygopus-1 Monoclonal Antibody (Catalog # MAB3617) followed by an HRP-conjugated Goat Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band for Pygopus-1 was detected at approximately 45 kDa (as indicated).



Detection of β-NGF-induced GSK-3α/β Phosphorylation using the DuoSet IC ELISA Development System. Lysates prepared from the PC12 rat adrenal pheochromocytoma cell line, uninduced or induced with Recombinant Rat β-NGF (Catalog # 556-NG) for 10 minutes, were assessed for GSK-3α/β phosphorylation using the Human/Mouse/Rat Phospho-GSK-3α/β (S21/S9) DuoSet IC ELISA Development System (Catalog # DYC2630; bar graph). The results obtained from the DuoSet IC ELISA were comparable to the relative levels of phosphorylated GSK-3α/β detected by Western blot (inset).