**R&D Systems** Cell Biology Focus
winter | 2006

# Cell Biology FOCUS

## Inside



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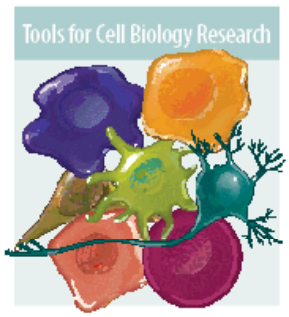


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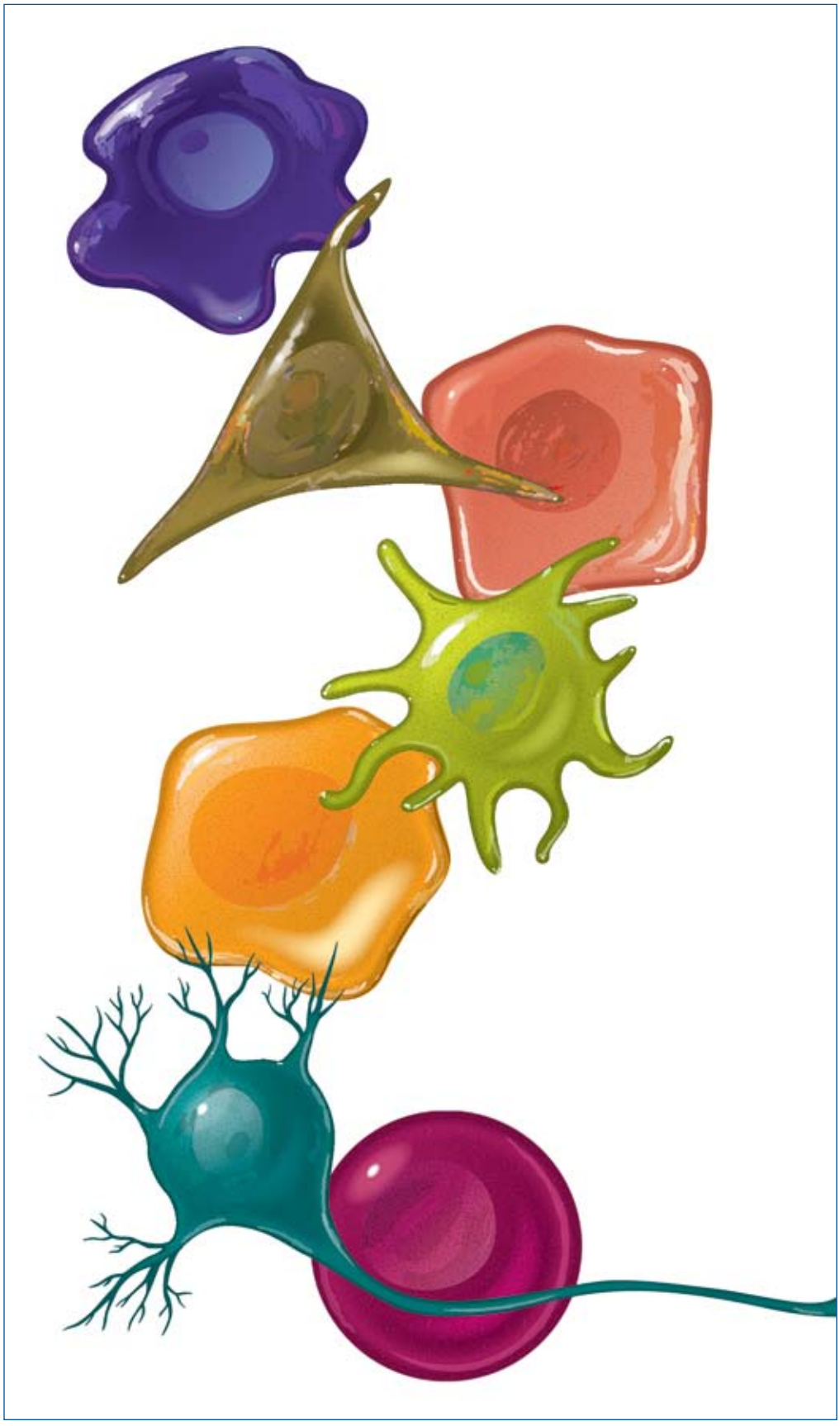

# Animal Lectins: Focus on Carbohydrate Binding

Lectins (from Latin Lectus, past participle of *legere*, **to select**) are carbohydrate-binding (**selecting**) proteins that show no enzyme activity towards sugars and are present in all life kingdoms. There are at least 13 groups (or families) of lectins in the animal kingdom, some with overlapping carbohydrate specificity. Among these are galectins, pentraxins, I- and P-type lectins, and C-type ($Ca^{2+}$-dependent) lectins. Also known as the C-type lectin domain (CTLD) superfamily, the C-type lectins include lecticans, type II transmembrane lectins, collectins, selectins, NK/lymphocyte receptors, and the macrophage mannose receptor. The table includes a list of research tools for animal lectins available from R&D Systems with the known (or proposed) carbohydrate specificities indicated. These reagents will be useful in defining the ligand specificities and additional functions of these lectins.

**Selected Lectin Products**

| MOLECULE | ANTIBODIES | PROTEINS | ELISAs | CARBOHYDRATE SPECIFICITY |
|---|---|---|---|---|
| Aggrecan | H | H | | Galactose/Fucose |
| ASGR1 | M | M | | Galactose/GalNAc |
| Calreticulin-2 | H | | | Glucose |
| CD44 | H | H | | Hyaluronan |
| CD48/SLAMF2 | M | H M | | Heparan Sulfate |
| CD83 | H M | M | | Sialic Acid |
| CL-P1/COLEC12 | H M | H M | | GalNAc |
| CLECSF13 | M | | | Galactose/Fucose |
| DC-SIGN | H | H | | Tri-Mannose/Fucose |
| DC-SIGNR/CD299 | H | H | | Tri-Mannose |
| Dectin-1/CLEC7A | H M | | | $(\beta 1,3\ Glucose)_n$ |
| Dectin-2/CLEC6A | H M | | | Mannose |
| Fcε RII/CD23 | H | H | | Galactose |
| Ficolin-2 | H | | | GlcNAc |
| Ficolin-3 | H | | | GlcNAc/GalNAc/Glucose |
| Galectin-1, -3 | H M | H M | M | Galactose/Lactose |
| Galectin-2, -8 | H | H | | Galactose/Lactose |
| Galectin-3 BP | H | | | Galactose/Lactose |
| Galectin-4 | H M | H | | Galactose/Lactose |
| Galectin-7 | H M | H M | H | Galactose/Lactose |
| Galectin-9 | H M | | | Galactose/Lactose |
| HAPLN1 | H | H | | Hyaluronan |
| ICAM-1/CD54 | H M R | H M R | H M R | Hyaluronan |
| IGF-II R | H | H | | Mannose |
| Langerin | H | | | Mannose/GlcNAc/Fucose |
| Layilin | H M | H M | | Hyaluronan |
| LSECtin/CLEC4G | H | | | Mannose/GlcNAc/Fucose |
| LYVE-1 | H M | H M | | Hyaluronan |

**Selected Lectin Products**

| MOLECULE | ANTIBODIES | PROTEINS | ELISAs | CARBOHYDRATE SPECIFICITY |
|---|---|---|---|---|
| MAG/Siglec-4a | R | R | | α2,3 Sialic Acid |
| MBL | H | H M | | GcNAc/Mannose/Fucose |
| MBL-2 | M | M | | GlcNAc/Mannose |
| MGL2 | M | M | | GalNAc |
| MMR | H M | H M | | Mannose/Fucose/GlcNAc |
| NCAM/CD56 | H | H | | Heparan Sulfate/ Chondroitin Sulfate |
| NCAM-L1 | H | H | | α2,3 Sialic Acid |
| OCIL/CLEC2d | H M | | | Sulfated Glycosaminoglycan |
| Pentraxin-3/TSG-14 | H M | H M | M | Mannan |
| Reg 2/PAP | R | | | Mannan |
| E-Selectin | H M R | H M R | H M | α2,3 Sialylated, Fucosylated Lactosamine |
| L-Selectin | H M R | H M R | H M R | α2,6 Sialic Acid |
| P-Selectin | H M | H M | H M | α2,6 Sialic Acid |
| Siglec-2/CD22 | H | H M | | α2,6 Sialic Acid |
| Siglec-3/CD33 | H M | H | | α2,6 Sialic Acid |
| Siglec-5, -9 | H | H | H | α2,3 Sialic Acid |
| Siglec-6 | H | H | | Sialic Acid/GlcNAc |
| Siglec-7 | H | H | H | α2,6 Sialic Acid |
| Siglec-10 | H | H | | α2,3 Sialic Acid |
| Siglec-11 | H | | | α2,8 Sialic Acid |
| Siglec-F | M | M | | 6-Sulfo-sLeX |
| SIGNR1/CD209 | M | M | | Mannan |
| SP-D | H | | | Maltose/Glucose/Mannose |
| TSG-6 | H M | | | Chondroitin Sulfate/ Hyaluronan |
| Versican | H | | | Hyaluronan |

Key: H Human M Mouse R Rat

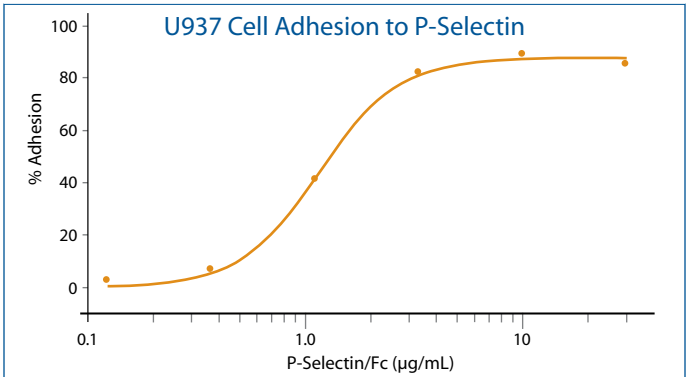


**Figure 1.** Microplate wells were coated with R&D Systems recombinant human P-selectin/Fc (Catalog # 137-PS) at the indicated concentrations. Recombinant P-selectin stimulates dose-dependent adhesion of the human histiocytic lymphoma cell line U937.


**Figure 2.** Galectin-3 was detected in a cryostat tissue section of embryonic mouse ribs (15 d.p.c.) using R&D Systems goat anti-mouse affinity purified galectin-3 antibody (Catalog # AF1197). Tissues were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

For our complete line of lectin-related products, please visit our website at **www.RnDSystems.com/go/Lectin**.

# Chemokine Receptors: Focus on Decoy Receptors

Chemokines regulate cell trafficking by binding and signaling via 7-transmembrane G protein-coupled receptors. However, there is a category of chemokine receptors termed decoy receptors that do not signal and do not mediate chemotaxis. Instead, they affect ligand internalization and degradation. Currently, there are three known decoy receptors in mammals and more than two dozen associated with viruses. Mammalian D6 only interacts with inflammatory chemokines of the CC subfamily, whereas CCXCKR binds hemostatic CC chemokines, and DARC binds both CXC and CC chemokines. Viral CCI is a soluble poxvirus-encoded molecule that binds to almost all CC chemokines. The effects of decoy receptors may be context-dependent. For example, while chemokine sequestration may inhibit cells from traveling into an area of inflammation, they may also block cells from leaving an area of inflammation. Additionally, DARC on endothelial cells is proposed to bind chemokines and transport them through the cell for presentation on the vessel cell surface where they are accessible to circulating leukocytes.

R&D Systems offers a variety of reagents to investigate this expanding field of research including chemokine-related antibodies, proteins, ELISAs, and tools for multi-analyte profiling. The table highlights antibodies that are currently available for chemokine receptor research.

For an updated listing of all chemokine-related products, please visit our website at **www.RnDSystems.com/go/Chemokine**.

## CCR1 Expression in Human Tonsil

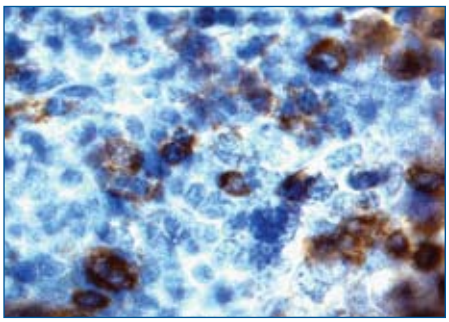

**Figure 1.** CCR1 was detected in a paraffin-embedded section of human tonsil using R&D Systems anti-human CCR1 monoclonal antibody (Catalog # MAB145). Tissues were stained using R&D Systems anti-mouse HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS002; brown) and counterstained with hematoxylin (blue).

**Chemokine Receptor-related Antibodies**

| MOLECULE | ANTIBODIES | MOLECULE | ANTIBODIES | MOLECULE | ANTIBODIES |
|---|---|---|---|---|---|
| APJ | H | CCR7 | H M | CXCR4 | H M |
| CCI | V | CCR8 | H | CXCR5 | H |
| CCR1 | H | CCR9 | H M | CXCR6 | H M |
| CCR2 | H | CCR10 | H M | D6 | H |
| CCR3 | H M | Chem R23 | H | HCR/CRAM-A/B | H |
| CCR4 | H | CXCR1/IL-8 RA | H | HM74A | H |
| CCR5 | H | CXCR2/IL-8 RB | H M | | |
| CCR6 | H M | CXCR3 | H M | | |

Key: H Human M Mouse V Viral

## Viral CCI Suppression of JE-mediated Chemotaxis & Inhibition by Anti-Viral CCI

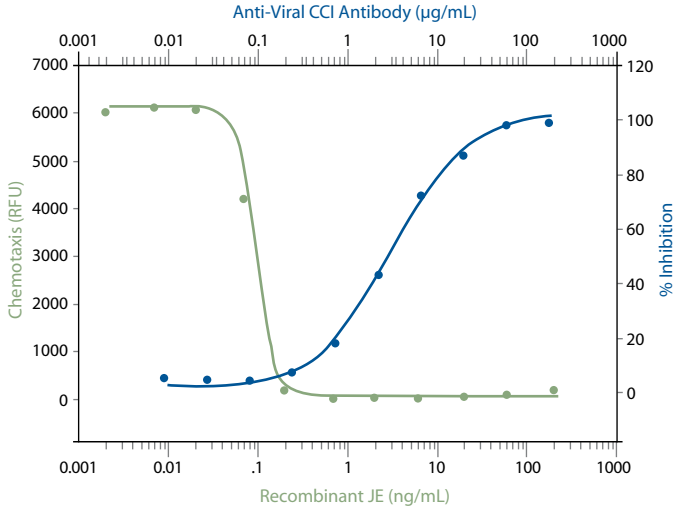


**Figure 2.** R&D Systems recombinant viral CCI (Catalog # 696-CC) inhibits the chemotaxis of CCR2-transfected BaF/3 cells toward 0.02 µg/mL of R&D Systems recombinant mouse JE (Catalog # 479-JE; green). The inhibitory effect of recombinant viral CCI (0.4 µg/mL) on JE-induced chemotaxis is neutralized by R&D Systems anti-viral CCI monoclonal antibody (Catalog # MAB6961; blue). Chemotaxis was assessed using a polycarbonate filter with a 5 µm pore size. Cells migrating through the filter were stained with resazurin (Catalog # AR002) and the relative fluorescence units (RFU) were measured using a spectrofluorometer.

## D6 Detection by Flow Cytometry

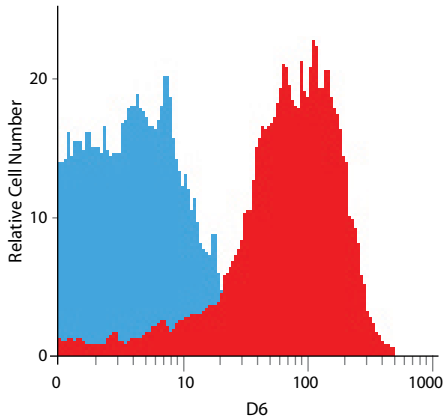


**Figure 3.** D6 was detected by flow cytometry in D6-transfected NS0 cells using R&D Systems anti-human phycoerythrin-conjugated D6 monoclonal antibody (Catalog # FAB 1364P; red histogram). Non-transfected control cells were also stained with the same antibody and assessed by flow cytometry (blue histogram).

# Epithelial-Mesenchymal Transition: Roles in Development & Cancer

The transition of cells from an epithelial to a mesenchymal phenotype (EMT) is an important morphogenetic mechanism during embryonic development. Gastrulation and neural crest formation both involve a transformation of cells from an epithelial state with strong cell-cell adhesion, apical-basal polarity, and limited migratory capacity, to a mesenchymal state characterized by decreased cell-cell adhesion, elongated irregular cell shape, and significant migratory ability. The molecular control of the EMT is being actively investigated. In adherent epithelial cells, β-catenin is sequestered in the cytoplasm by participation in a complex with the adhesion proteins E-cadherin and α-catenin. HGF, acting through c-met/HGF receptor, phosphorylates β-catenin, leading to its dissociation from the E-cadherin complex and entry into the nucleus. In addition, Wnt ligands control the amount of β-catenin available in the cytoplasm by preventing its ubiquitin-mediated degradation. Additional autocrine factors that have been shown to promote EMT include TGF-β, EGF family members, IGF-I, and IGF-II. EMT is also of interest due to a putative role in cancer, where it can allow cells from solid tumors to acquire a migratory phenotype conducive to metastasis.

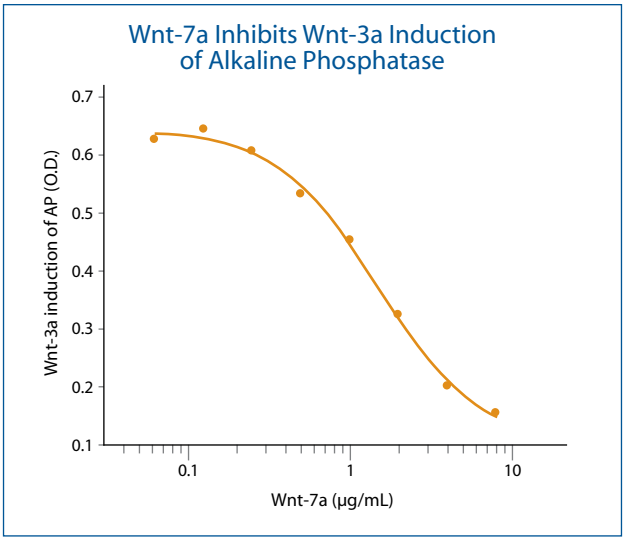


**Figure 1.** Wnt-7a inhibits the ability of Wnt-3a to induce alkaline phosphatase (AP) synthesis by MC3T3-E1 osteoblastic cells. Cells were treated with increasing concentrations of R&D Systems recombinant human Wnt-7a (Catalog # 3008-WN) in the presence of 10 ng/mL R&D Systems recombinant mouse Wnt-3a (Catalog # 1324-WN) for three days.

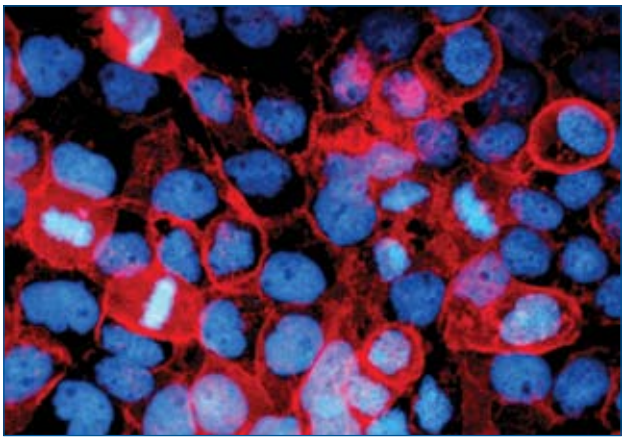

**Figure 2.** E-cadherin was detected at the junctions of human epidermoid carcinoma cells using R&D Systems goat anti-human E-cadherin affinity purified polyclonal antibody (Catalog # AF648). Cells were stained with R&D Systems NorthernLights™-547 anti-goat IgG secondary antibody (Catalog # NL004; red) and counterstained with DAPI (blue).

**Epithelial-Mesenchymal Transition-related Products**

| MOLECULE | ANTIBODIES | PROTEINS | ELISAs |
|---|---|---|---|
| β-Catenin | H M R X | | H |
| Claudin-6 | H | | |
| Cytokeratin 8 | H | | |
| Dkk-1 | H M | H M | H M |
| Dkk-2 | M | | |
| Dkk-3 | H M | H | |
| Dkk-4 | H M | H M | |
| E-Cadherin | H M | H M | H M |
| N-Cadherin | | H | |
| EGF | H M | H M R | H M |
| EGF R | H M | H | H |
| Fibronectin | H | H B | |
| Frizzled-1 | H M | M | |
| Frizzled-2, -8 | M | M | |
| Frizzled-3, -6 | H M | | |
| Frizzled-4, -7 | H M | M | |
| Frizzled-5 | H | H | |
| Frizzled-9 | M | | |
| Glypican 3 | H | H | |
| Glypican 5 | H M | H M | |
| GSK-3α | H | | |
| GSK -3α/β, -3β | H M R | | H M R |
| HGF | H M | H M Ca | H |
| HGF R | H M | H M | H M |
| IGF-I | H M | H M | H M |
| IGF-II | H M | H M | M |
| Kremen-1 | H M | M | |
| Kremen-2 | H M | H M | |
| LRP-1 | H | | |
| LRP-6 | H M | H M | |
| Pygopus-1 | H M | | |
| Pygopus-2 | H | | |
| R-Spondin 1 | M | | |
| R-Spondin 2, 3 | H | | |
| sFRP-1, -4 | H | H | |
| sFRP-2 | M | M | |
| sFRP-3 | H M | H M | |
| TGF-β Superfamily | See our website for our wide range of TGF-β superfamily research reagents. | | |
| Vimentin | H | | |
| Vitronectin | | H B | |
| Wnt-1, -4, -8a, -9b, -10b, -11 | M | | |
| Wnt-3a, -5a | M | M | |
| Wnt-5b | | M | |
| Wnt-7a | H | H | |
| Wnt-7b, -9a | H | | |

Key: H Human M Mouse R Rat B Bovine Ca Canine X *Xenopus*

# Cell Cycle Checkpoints

The proper timing and order of cell cycle events is critical to ensure the faithful replication of DNA to two daughter cells. The mammalian cell cycle is divided into a DNA synthesis (S) phase and a cell division or mitosis (M) phase, separated by two gap or growth phases (G1 and G2). These events are enforced by cell cycle checkpoints at each transition stage (G1 to S, S to G2, G2 to M) and within different stages of the cell cycle.

Cell cycle checkpoints are enforced by a number of different protein kinases and adaptor molecules. These checkpoint proteins act to ensure accuracy during the normal cell cycle, and many also act in response to genotoxic stress from endogenous or environmental sources. Endogenous sources of DNA damage result from cellular metabolism or routine errors in DNA replication and recombination. In the case of genotoxic stress, the cell cycle must be halted until DNA repair occurs and the cycle can resume.

## Phospho-p53-induction by Camptothecin

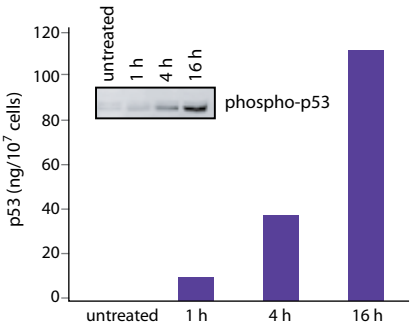


**Figure 1.** MCF-7 cells were left untreated or treated with 1 µM camptothecin (CPT). Cellular extracts were prepared at the indicated times following CPT treatment. Phosphorylated p53 was quantified using R&D Systems phospho-p53 (S15) DuoSet® IC ELISA (Catalog # DYC1839). The same cellular extracts were immunoblotted (inset) using R&D Systems rabbit anti-phospho-p53 (S15) affinity-purified polyclonal antibody (Catalog # AF1043). The DuoSet IC results correlate well with the amounts of phospho-p53 (S15) detected by Western Blot.

## Phospho-p53 (S15) Detected by Flow Cytometry

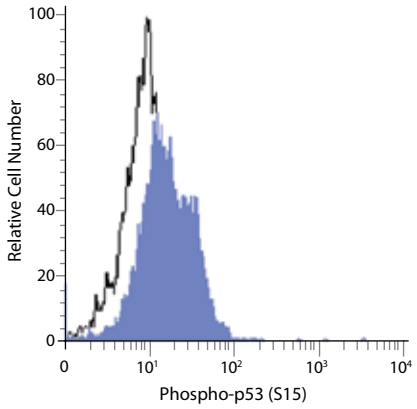


**Figure 2.** Phospho-p53 (S15) was detected by flow cytometry in camptothecin-stimulated MCF-7 cells using R&D Systems allophycocyanin-conjugated anti-human phospho-p53 (S15) antibody (Catalog # IC18392A; filled histogram). Control cells were also stained with R&D Systems isotype control antibody (Catalog # IC003A, open histogram).

**Cell Cycle/Checkpoint-related Products**

| MOLECULE | ANTIBODIES | PROTEINS | ELISAs |
|---|---|---|---|
| 53BP1 | H | | |
| ATM | H M R | | H |
| ATRIP | H M R | | |
| Aurora A | H | | |
| BARD1 | H M R | | |
| BRCA1 | H M R | | |
| BRCA2 | H | | |
| CBP | H M R | | |
| CDC2 | H M R | | |
| CDC25A | H M R | H | |
| CDC25B | H M R | H | |
| Chk1 | H M R | | H M R |
| Chk2 | H M R | | H M R |
| Claspin | H | | |
| H2AX | H | | |
| MDM2 | H M R | | |
| Mre11 | H | | |
| Nbs1 | H M R | | |
| p21/CIP1/CDKN1A | H | | H |
| p27/Kip1 | H M R | | H |
| p53 | H M R | | H M |
| Pin1 | H M | H | |
| PLKK | X | | |
| Rad1 | H | | |
| Rad17 | H R | | H |
| SMC1 | H M R | | |
| Wip1 | H | | |

Key: H Human M Mouse R Rat X *Xenopus*

For our complete line of products for Cell Cycle and Genotoxic Stress, please visit our website at **www.RnDSystems.com/go/CellCycle**.

## Inhibition of Chk1 Kinase Activity

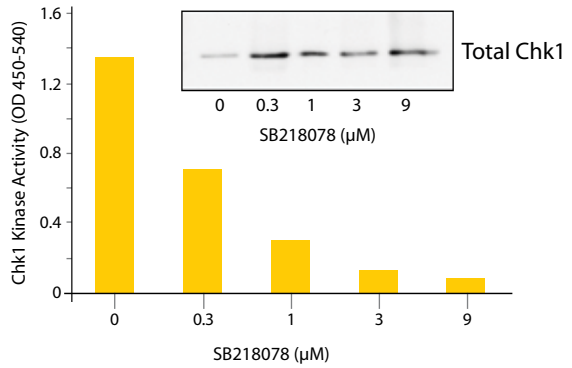


**Figure 3.** Chk1 kinase activity in HeLa cell lysates was assayed in the presence of increasing concentrations of Chk1-specific inhibitor SB218078 using R&D Systems Human/Mouse/Rat Active Chk1 DuoSet IC IP Kinase Assay Kit (Catalog # DYC1630). Western blotting using R&D Systems goat anti-human/mouse/rat Chk1 affinity purified polyclonal antibody (Catalog # AF1630) was used to confirm equal amounts of Chk1 protein in the immunoprecipitates (inset).

# ITIM/ITAM Immunoreceptors

ITAMs (immunoreceptor tyrosine-based activation motif; consensus sequence YxxI/$Lx_{6-12}$YxxI/L) and ITIMs (immunoreceptor tyrosine-based inhibition motif; S/I/V/LxYxxI/V/L) are phosphorylation motifs found in a large number of receptors or adaptor proteins. Phosphorylated ITAMs serve as docking sites for tandem SH2 domains of Syk family kinases, whereas phosphorylated ITIMs recruit tyrosine phosphatases. Signaling through ITAM-bearing receptors usually results in cell activation, while engagement of ITIM-bearing receptors is usually inhibitory, although exceptions have been described. The majority of these receptors are involved in tumor development and regulation of the immune system, although some also function in tissues such as bone and brain.

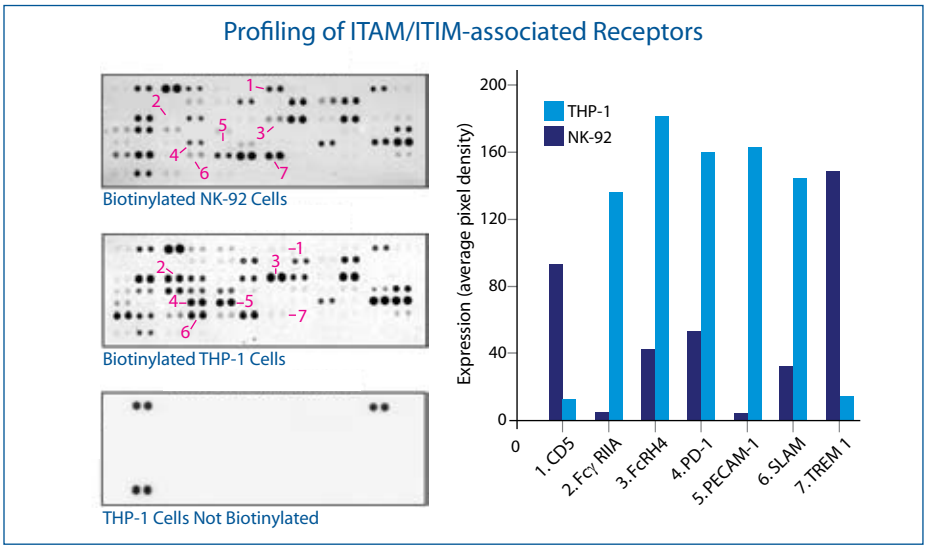


**Figure 1.** R&D Systems Proteome Profiler™ Phospho-Immunoreceptor Array Kit (Catalog # ARY004) is a multifunctional tool for simultaneously measuring the relative phosphorylation of 59 ITAM/ITIM-associated immunoreceptors, or obtaining a total immunoreceptor profile using lysates prepared from cell-surface biotinylated cells. Cell surface proteins were biotinylated on the cell lines NK-92 (human NK cells) and THP-1 (human monocytic cells). Arrays with capture antibodies spotted in duplicate were incubated with the lysates and the levels of bound immunoreceptors assessed with streptavidin-HRP and chemiluminescence detection. The numbered arrows and the accompanying histogram highlight several proteins exhibiting differential expression. The bottom array was incubated with THP-1 lysates without prior cell surface protein biotinylation and only positive control spots are seen.

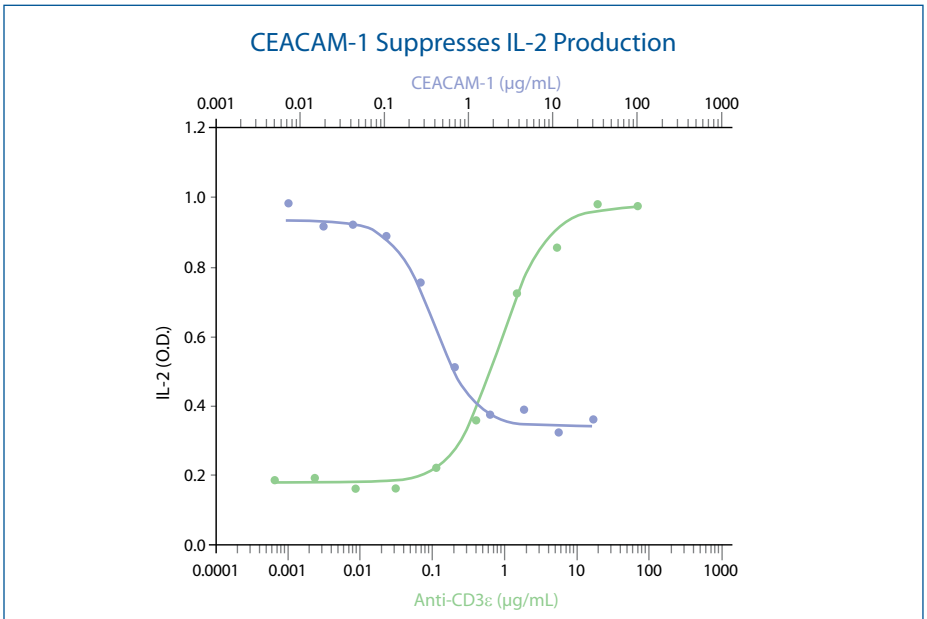


**Figure 2.** HuT-78 cells (human T cell line) were cultured in microplates coated with R&D Systems anti-human CD3ε monoclonal antibody (Catalog # MAB100), or anti-CD3ε and R&D Systems recombinant human CEACAM-1 (Catalog # 2244-CM). Anti-CD3ε alone stimulates a dose-dependent increase in the levels of IL-2 in the culture supernantant as measured using R&D Systems Quantikine Human IL-2 ELISA Kit (Catalog # D2050; green line). The stimulatory effect of anti-CD3ε (10 µg/mL) is inhibited by increasing concentrations of recombinant CEACAM-1 (purple line).

**ITIM/ITAM Immunoreceptor-related Products**

| MOLECULE | ANTIBODIES | PROTEINS |
|---|---|---|
| 2B4/SLAMF4/CD224* | H M | M |
| BLAME/SLAMF8* | H | |
| BTLA* | H M | |
| CD3ε* | H M | |
| CD5* | H M | |
| CD6* | H M | H M |
| CD28* | H M | H M |
| CD72 | M | |
| CD84/SLAMF5* | H | H |
| CD155/PVR | H | H |
| CD200 R1 | M | H |
| CD229/SLAMF3* | H M | |
| CEACAM-1/CD66a* | H | H |
| CLEC-1*, 2 | H | |
| CRACC/SLAMF7* | H | |
| CTLA-4* | H M | H M |
| DCIR/CLEC4A* | H M | |
| Dectin-1/CLEC7A* | H M | |
| DNAM-1* | H | H |
| FcγRI, RIIA*, RIIB, RIII* | H M | H M |
| FcγRIIC | H | H |
| FcεRII* | H | H |
| FcRH1*, 2*, 4*, 5* | H | |
| ILT/LIR Family* Please see our website for a detailed product listing | | |
| Integrin $\beta_2$/CD18 | H M | |
| Integrin $\beta_3$/CD61* | H | |
| KIR/CD158 | H | |
| KIR2DL1, KIR2DL3 | H | |
| KIR2DL4/CD158d* | H | H |
| KIR2DS4 | H | |
| KIR3DL1 | H | |
| KIR3DL2 | | H |
| LAIR1*, LAIR2* | H | |
| LMIR Family* Please see our website for a detailed product listing | | |
| MDL-1/CLEC5A* | H M | |
| NKp30, NKp44* | H | H |
| NKp46/NCR1* | H M | H M |
| NKp80/KLRF1* | H | |
| NTB-A/SLAMF6* | H | |
| PD-1* | H M | H M |
| PECAM-1/CD31* | H | H M P |
| Siglec Family* Please see page 2 of this Newsletter | | |
| SLAM* | H | |
| TREM Family* Please see our website for a detailed product listing | | |

Key: H Human M Mouse P Porcine

* ITAM/ITIM-associated receptors included in the Human Phospho-Immunoreceptor Array (Catalog # ARY004).

# Akt and Insulin Signaling

The phosphatidylinositol 3-kinase (PI3-K)/Akt signaling pathway is responsible for many of the metabolic actions of insulin. In this pathway, ligand-bound insulin receptor phosphorylates insulin receptor substrate (IRS) proteins, which in turn activate PI3-K. Members of the Akt family are classical effectors of PI3-K and include Akt1 (also known as PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). Of the three Akts, Akt2 appears most tightly associated with insulin signaling. Active PI3-K generates the second messenger phosphatidylinositol-3,4,5-trisphosphate ($PIP_3$), which recruits Akt to the plasma membrane for subsequent phosphorylation by phosphoinositide-dependent kinase-1 (PDK-1). PDK-1 phosphorylates Akt in the activation loop of the kinase domain (T309 of Akt2), and an unknown kinase, possibly the rictor/mTOR complex, phosphorylates Akt in the regulatory domain (S474 of Akt2). Phosphorylation at both sites is necessary for full Akt activation.

Signaling circuits regulated by active Akt mediate insulin's downstream effects by increasing protein synthesis, altering the cytoskeleton, and modifying gene expression. For example, mTOR activation by Akt in turn activates p70 S6 kinase and inactivates 4E-BP1, upregulating protein translation. Also, Akt's inhibitory phosphorylation of AS160 (Akt substrate of 160 kDa) triggers the activation of Rab small GTPases involved in cytoskeletal reorganization, promoting the translocation of glucose transporter GLUT4 to the plasma membrane. Additionally, Akt-mediated phosphorylation of FOX class transcription factors leads to their interaction with 14-3-3 proteins and prevents their nuclear localization, inhibiting gluconeogenic transcription and activating adipogenesis.

**Akt/Insulin-associated Products**

| MOLECULE | PROTEINS | ANTIBODIES | ELISAs |
|---|---|---|---|
| 14-3-3ζ | H | H M | |
| 4EBP1 | | H M | |
| Akt1 | | H M R | H M R |
| Akt2 | | H M R | |
| Akt3 | | H | |
| ASK1 | | H | |
| Bad | | H M | H M R |
| β-Catenin | | H M R X | H |
| Chk1 | | H M R | H M R |
| FoxD3 | | H | |
| FoxJ1 | | H | |
| FoxP3 | | H | |
| Glut4 | | R | |
| GSK-3α | | H | |
| GSK-3β | | H M R | H M R |
| IGF-I | H M | H M | H M |
| IGF-I R | H | H | H |

| MOLECULE | PROTEINS | ANTIBODIES | ELISAs |
|---|---|---|---|
| IKKα | | H M R | |
| IKKγ | | H M R | |
| IKKε | | H M R | |
| Insulin | | H M B | |
| Insulin R/CD220 | H | H | H |
| MDM2 | | H M R | |
| p27/Kip1 | | H M R | H |
| p70 S6 Kinase | | H M R | H M R |
| PDK-1 | | H | |
| PI 3-Kinase p85α | | H M R | |
| PI 3-Kinase p110β | | H | |
| PI 3-Kinase p110γ | | H | |
| PI 3-Kinase p110δ | | H | |
| PP2A | | H M R | H M R |
| PTEN | H | H M R | H M R |
| TOR | | H M R | H |
| WNK1 | | M R | |

Key: H Human M Mouse R Rat B Bovine X *Xenopus*

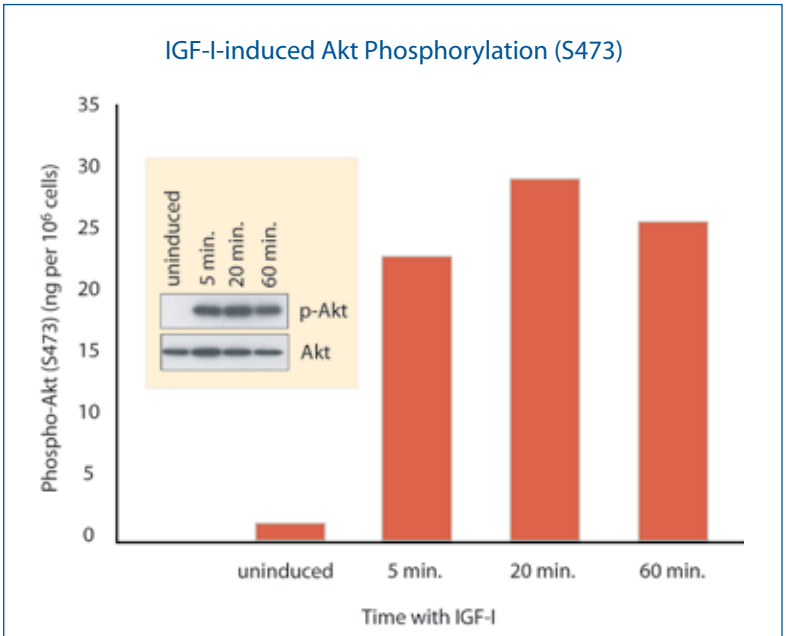


**Figure 1.** Lysates prepared from MCF-7 cells and induced with IGF-I for the indicated times were quantified using R&D Systems Phospho-Akt (S473) pan specific DuoSet IC ELISA (Catalog # DYC887). The same lysates were also immunoblotted (inset) using either R&D Systems anti-phospho Akt (S473) pan specific (p-Akt) (Catalog # AF887) or anti-Akt pan specific (Catalog # MAB2055) antibodies. The results using the DuoSet IC ELISA correlate well with the amounts of phosphorylated Akt detected by Western blot. The immunoblot with anti-Akt antibody indicates that total levels of Akt remained constant during the induction with IGF-I.

Akt1 Detection by Flow Cytometry

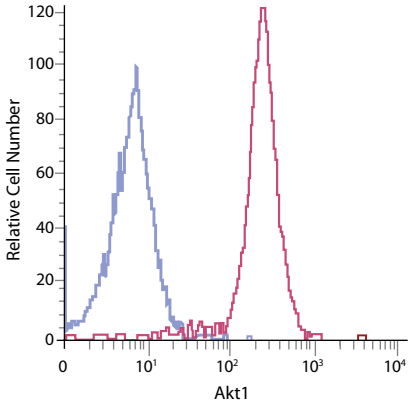


**Figure 2.** Jurkat T cells were fixed, permeabilized, and incubated with R&D Systems anti-human Akt1 monoclonal antibody (Catalog # MAB17751). Cells were then stained with R&D Systems anti-mouse IgG NorthernLights-637 secondary antibody (Catalog # NL008; magenta histogram). Control staining was done by incubating cells in R&D Systems IgG1 isotype control monoclonal antibody (Catalog # MAB002), followed by staining with NorthernLights-637 (purple histogram).

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NorthernLights fluorochrome-conjugated antibodies provide an intense fluorescent signal that is resistant to photobleaching. Like all R&D Systems antibodies, NorthernLights antibody conjugates are highly specific and deliver a high signal-to-noise ratio. We are currently offering antibodies recognizing mouse, goat, and rabbit IgG, as well as a streptavidin conjugate for labeling biotinylated antibodies. These secondary reagents are available with three distinct excitation and emission maxima, making them ideal for multicolor fluorescence microscopy (Figure 1). The spectral properties of these antibodies are similar to several frequently used fluorochromes making them suitable for use with common filter sets and/or lasers (Table). NorthernLights secondary antibodies are stable when exposed to alcohols and xylene, as well as DPX mounting medium (a mixture of distyrene, a plasticizer, and xylene). They have been tested for their suitability in both immunochemistry and flow cytometry (Figure 2).

| NorthernLights Antibody/Label | Catalog # | Abs/Em Maxima | Laser (Ex) | Comparable Fluorochromes |
|---|---|---|---|---|
| NL-493 anti-Rabbit IgG<br>NL-493 anti-Mouse IgG<br>NL-493 anti-Goat IgG<br>NL-493 Streptavidin | NL006<br>NL009<br>NL003<br>NL997 | 493/514 | Argon (488) | FITC (492/520)<br>Cy™2 (489/506)<br>Alexa Fluor® 488 (494/519) |
| NL-557 anti-Rabbit IgG<br>NL-557 anti-Mouse IgG<br>NL-557 anti-Goat IgG<br>NL-557 Streptavidin | NL001<br>NL007<br>NL004<br>NL999 | 557/575 | Krypton (568)<br>HeNe (543) | Phycoerythrin (565/575)<br>Rhodamine Red X (570/590)<br>Cy™3 (548/562) |
| NL-637 anti-Rabbit IgG<br>NL-637 anti-Mouse IgG<br>NL-637 anti-Goat IgG<br>NL-637 Streptavidin | NL005<br>NL008<br>NL002<br>NL998 | 637/658 | HeNe (633) | Allophycocyanin (645/660)<br>Alexa Fluor® 647 (650/668)<br>Cy™5 (650/670) |

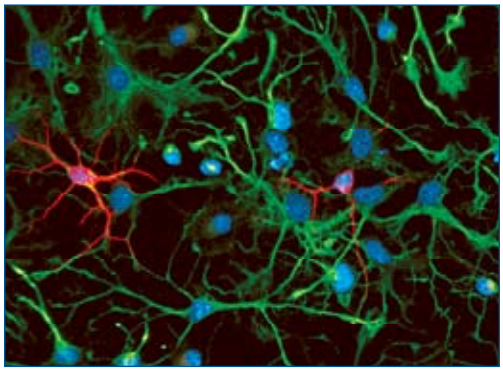

**Figure 1.** Rat cortical stem cell differentiation was monitored using multicolor immunocytochemistry. Neural progenitors were labeled with R&D Systems goat anti-rat nestin polyclonal antibody (Catalog # AF2736) and stained with R&D Systems donkey anti-goat Northern Lights-493 secondary antibody (Catalog # NL003; green). Differentiated neurons were labeled with R&D Systems neuron-specific mouse anti-β-III tubulin monoclonal antibody (TuJ1; Catalog # MAB1195) and stained using R&D Systems donkey anti-mouse Northern Lights-557 secondary antibody (Catalog # NL007; red). Nuclei were stained with DAPI (blue).

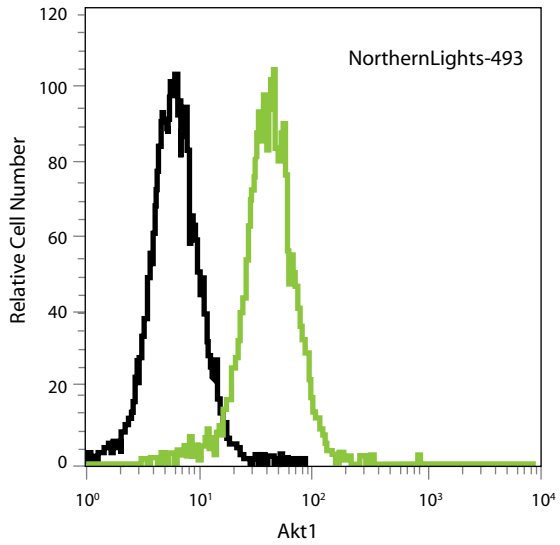


**Figure 2.** Jurkat T cells were fixed, permeabilized, and incubated with R&D Systems anti-human Akt1 monoclonal antibody (Catalog # MAB17751). Cells were then stained with R&D Systems anti-mouse IgG NorthernLights-493 (Catalog # NL009; green histogram). Control staining was done by incubating cells in R&D Systems IgG1 isotype control monoclonal antibody (Catalog # MAB002), followed by staining with NorthernLights-493 (black histogram).





Quality | Selection | Performance | Results

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