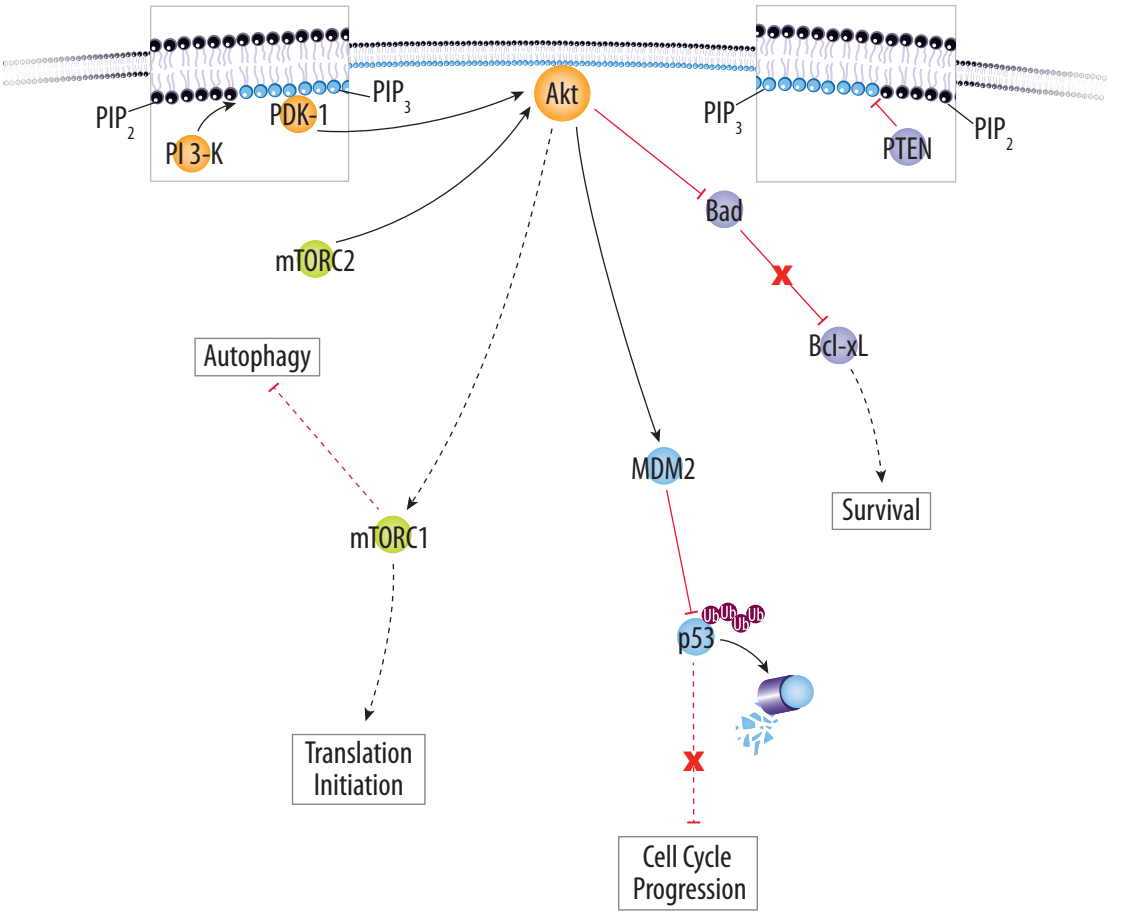
PI 3-Kinase/Akt Signaling Pathways



R&D SYSTEMS®

PI 3-Kinase/Akt Signaling Pathways

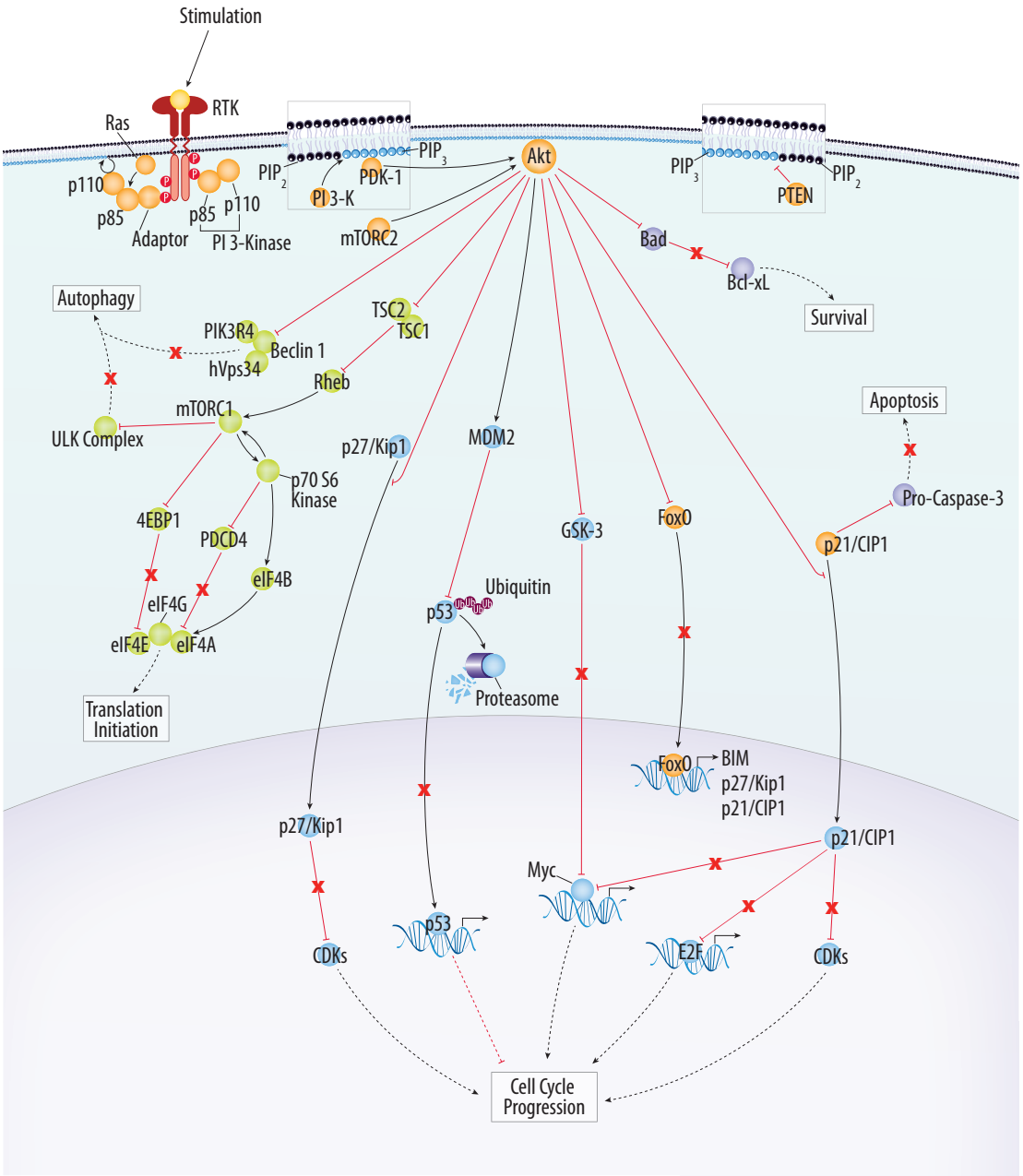
The Akt pathway is activated in response to growth factors and regulates many cellular processes, including protein synthesis, cell survival, proliferation, autophagy, and metabolism. Akt is a three-member family of serine-threonine protein kinases consisting of Akt1, Akt2, and Akt3. The pathway is classically activated downstream of PI 3-Kinase-dependent phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) formation at the plasma membrane. Conversely, Akt activation is negatively regulated by the lipid phosphatase PTEN, which dephosphorylates PIP_3.[1] Due to its role in the promotion of protein synthesis, cell survival and proliferation, dysregulation of the Akt pathway can promote tumorigenesis.[2] Akt is also known to suppress autophagy, which can either promote or inhibit cancer cell death in a context-dependent manner.[3] In addition, Akt deficiency is associated with the development of diabetes in mice and humans, suggesting the pathway is also important for proper regulation of metabolism.[4] The association of unregulated Akt signaling in multiple diseases highlights the need for more research and a better understanding of the pathway and its regulation.

R&D Systems offers a wide range of proteins, antibodies, ELISAs, multianalyte profiling kits, and small molecule inhibitors for studying PI 3-Kinase/Akt signaling.

To view related articles and up-to-date product listings, please visit **www.RnDSystems.com/AktPathway**

References

1. Song, M.S. *et al.* (2012) Nat. Rev. Mol. Cell Biol. **13**:283.
2. Lui, P. *et al.* (2009) Nat. Rev. Drug Discov. **8**:627.
3. White, E. (2012) Nat. Rev. Cancer **12**:401.
4. Hay, N. (2011) Trends Endocrinol. Metab. **22**:66.



Survey multiple pathways for activation to obtain more information.

Antibody Arrays save time, are cost-effective, and provide an unbiased approach to your research by allowing for the simultaneous visualization of multiple interrelated pathways. Choose from either membrane-based or microplate-based formats to meet your research needs.

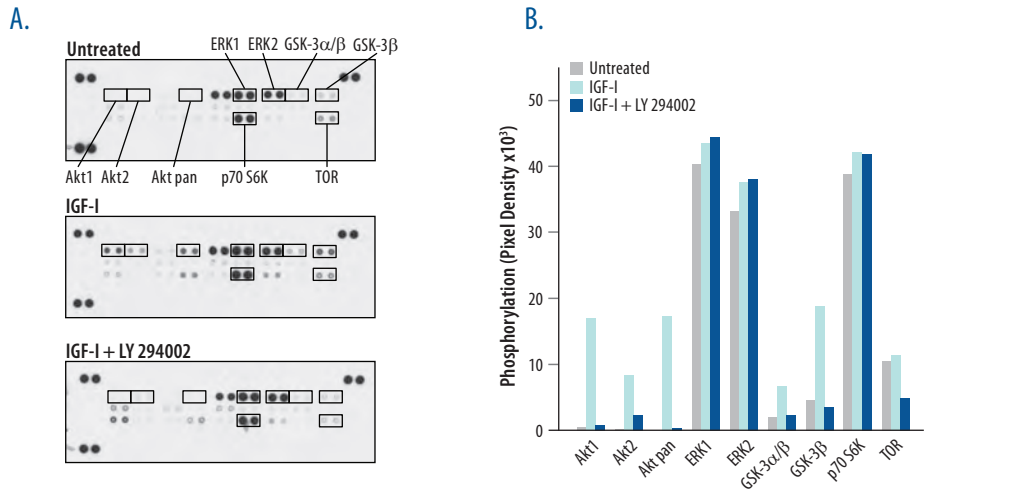
Proteome Profiler™ Phospho-specific Arrays: Membrane-based Profiling

These nitrocellulose membrane-based arrays are spotted in duplicate with a range of capture antibodies. The chemiluminescent-based detection method does not require specialized equipment beyond what is typically used to collect Western blot data. In addition, they are easier to perform than a Western blot and are ideal for rapidly providing a more complete picture of protein phosphorylation by allowing you to simultaneously profile multiple pathways.

FEATURES

- ✓ Most cited membrane-based phospho-protein array
- ✓ Ideal for visualizing signaling crosstalk
- ✓ Easier to perform than a Western blot
- ✓ Hands-on time of 3 hours

To view additional information about Proteome Profiler Antibody Arrays, please visit **www.RnDSystems.com/ProteomeProfiler**



Intracellular Signal Transduction in Response to IGF-I Treatment. MCF-7 human breast cancer cells were either untreated or treated with 100 ng/mL of Recombinant Human IGF-I (Catalog # 291-G1) for 1 hour. Cells treated with IGF-I either received a 1 hour pre-treatment with the PI 3-Kinase inhibitor, LY 294002 (Catalog # 1130), or were untreated. The phosphorylation status was determined using the Proteome Profiler Human Phospho-MAPK Array (Catalog # ARY002B). Membranes were exposed to X-ray film (A) and histograms were generated from pixel density measurements (B).

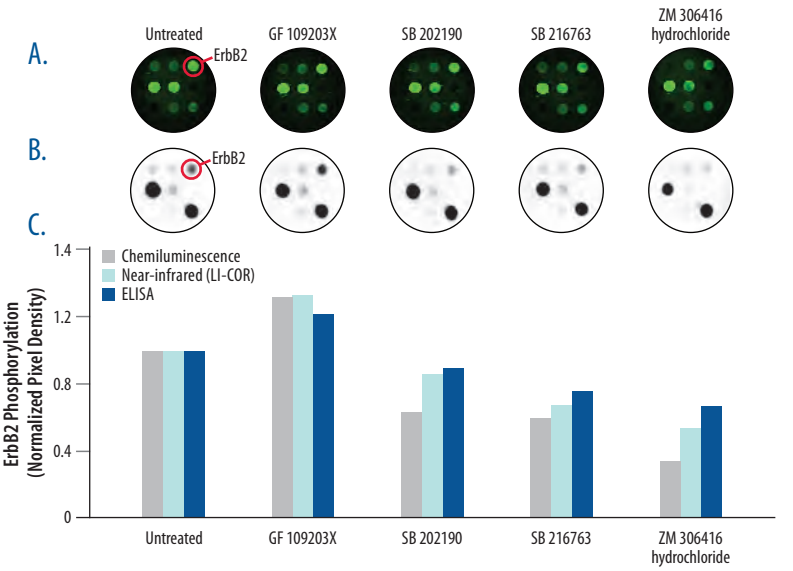
Proteome Profiler *96* Phospho-specific Arrays: Microplate-based Profiling

Phosphorylation can be determined using either pre-defined or custom-made Proteome Profiler *96* Phospho-Protein Antibody Arrays. These microplate-based arrays are well suited for high-throughput screening. Common imaging systems are used for data acquisition.

FEATURES

- ✓ An array in each well of a 96-well microplate
- ✓ Acquire up to 1,536 data points per microplate
- ✓ Choose chemiluminescence or LI-COR® detection
- ✓ Order pre-defined or custom arrays
- ✓ Compatible imaging systems include: Quansys Biosciences Q-View™, FujiFilm LAS-3000 or LAS-3000 Mini, BioRad® VersaDoc™ 4000, BioRad® ChemiDoc™ XRS, Alpha Innotech FluorChem® HD2, Alpha Innotech FluorChem® FC2, and Aushon BioSystems SearchLight®

To view additional information about Proteome Profiler *96* Antibody Arrays, please visit **www.RnDSystems.com/ProteomeProfiler96**



Near-infrared and Chemiluminescent Detection used to Detect ErbB2 Phosphorylation in an Inhibitor Screen. MDA-MB-453 human breast cancer cells were treated for 4 hours with 50 µM of GF 109203X (Catalog # 0741; PKC Inhibitor), SB 202190 (Catalog # 1264; p38 Inhibitor), SB 216763 (Catalog # 1616; GSK3 Inhibitor), or ZM 306416 hydrochloride (Catalog # 2499; VEGF R Inhibitor). Phosphorylation of ErbB2 was assessed in cell lysates by: **(A)** near-infrared, LI-COR® -based detection using the Proteome Profiler *96* Human Phospho-RTK Array 2 NIR (Catalog # ARZ002NIR); **(B)** chemiluminescent detection using the Proteome Profiler *96* Human Phospho-RTK Array 2 (Catalog # ARZ002); and **(C)** with the Human Phospho-ErbB2 DuoSet® IC ELISA (Catalog # DYC1768). Results obtained using all three detection methods were comparable.

Generate 8 Western blots worth of data in a fraction of the time by using an ELISA.

R&D Systems offers three ELISA-based formats that allow you to optimize your experimental conditions more quickly. Our Cell-Based ELISA Kits, Surveyor™ IC ELISA Kits, and DuoSet® IC ELISA Development Systems utilize a 96-well microplate format that can provide as much data as approximately eight individual Western blots. Therefore, dose-response experiments and inhibitor screens in multiple cell types can be accomplished using a single kit.

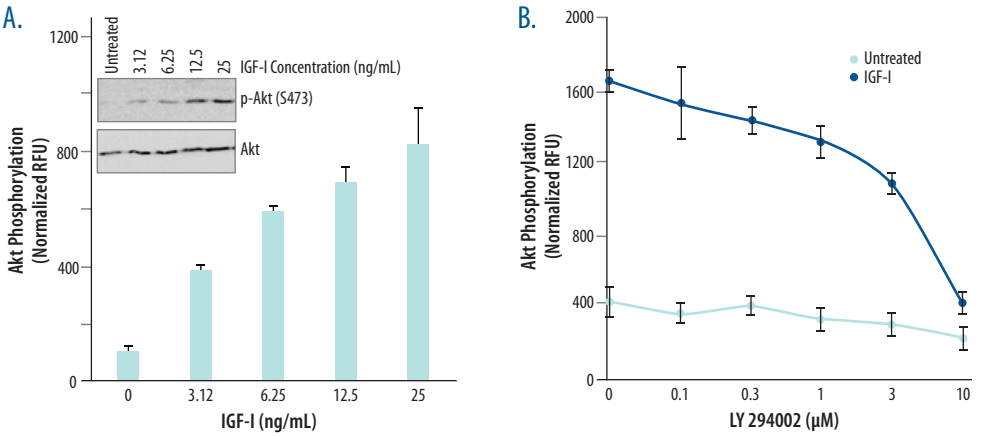
Cell-Based ELISA Assays: Analysis of Intact Cells

Cell-Based ELISA assays measure phosphorylation in fixed cells and do not require lysate preparation. The cells are cultured and the assay is run in the same plate. The data obtained with a fluorescence plate reader can be compared across multiple samples because it is normalized using the ratio of phosphorylated and total protein in each well.

FEATURES

- ✓ Obtain data from intact cells
- ✓ Utilize with either adherent or suspension cells
- ✓ Measure the levels of phosphorylated and total protein in the same well
- ✓ Culture cells and perform the assay in the same well
- ✓ Begin with as few as 10,000 cells per well

To view additional information about R&D Systems ELISA Assays, please visit **www.RnDSystems.com/ELISA**



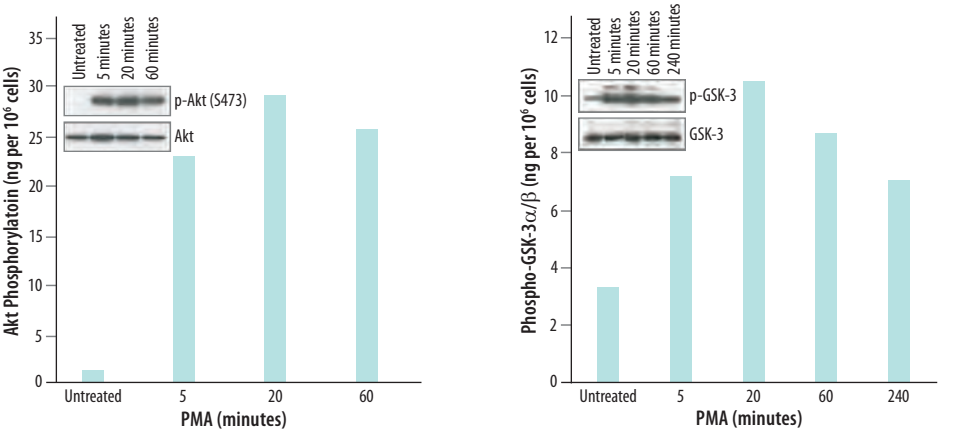
Measurement of Akt Phosphorylation in MCF-7 Cells. MCF-7 human breast adenocarcinoma cells were treated with increasing concentrations of Recombinant Human IGF-I (Catalog # 291-G1) for 20 minutes **(A)**, or were pretreated for 10 minutes with the indicated concentrations of the PI 3-Kinase inhibitor LY 294002 (Catalog # 1130) and then incubated with no additions or with 25 ng/mL IGF-I for 20 minutes. Phosphorylated Akt (S473) and total Akt were also detected by Western blot (inset) using the antibodies supplied in the kit. **(B)** After fixation of cells in the wells, phospho-Akt (S473) levels were determined and normalized to total Akt levels in the same well using the Human/Mouse/Rat Phospho-Akt (S473) Pan Specific Cell-Based ELISA (Catalog # KCB887).

Surveyor™ IC (Intracellular) ELISA Assays and DuoSet® IC ELISA Development Systems

Surveyor IC ELISA Kits are complete assays that provide all of the components necessary for performing a successful sandwich ELISA. DuoSet IC ELISA Development Systems allow you to customize an assay specifically for your needs by providing validated matched antibody pairs, buffers, and protein standards for the development of your assay.

FEATURES

- ✓ Benefit from the sensitivity and specificity of a sandwich ELISA
- ✓ Obtain the same amount of data in one plate as in 8 Western blots
- ✓ Acquire dose-response, time-course and pharmacology data in a single plate

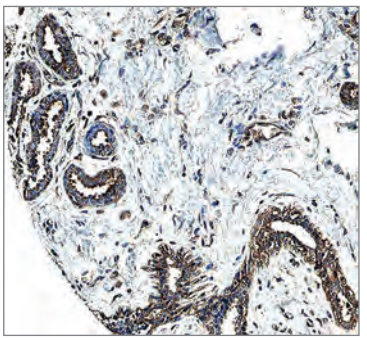


Quantification of Phospho-Akt in MCF-7 Cells. MCF-7 human breast adenocarcinoma cells were treated with Recombinant Human IGF-I (Catalog # 291-G1) for the indicated times. The levels of phospho-Akt (S473) were quantified using the Phospho-Akt (S473) Pan Specific Surveyor IC Kit (Catalog # 887) and by Western blot (inset).

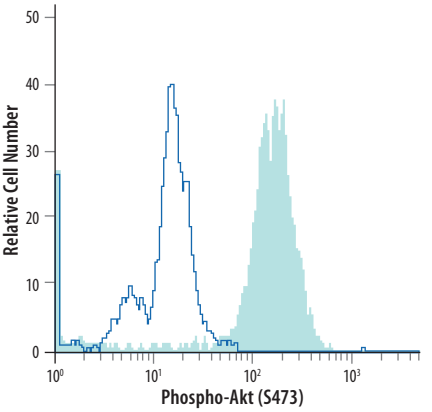
Quantification of Phospho-GSK-3α/β in HeLa Cells. HeLa human cervical epithelial carcinoma cells were induced with 200 nM PMA (Catalog # 1201). The levels of phospho-GSK-3α/β (S21/S9) were quantified using the Phospho-GSK-3α/β (S21/S9) DuoSet IC Kit (Catalog # DYC2630) and by Western blot (inset).

Test your hypothesis with high performance signal transduction antibodies.

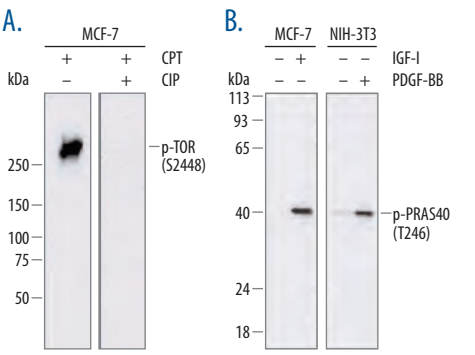
The data obtained with R&D Systems industry leading arrays and ELISA kits can help shape hypotheses and optimize experimental conditions. We also provide high-quality antibodies to test your hypotheses with confidence. R&D Systems offers a wide range of Akt signaling-related antibodies for flow cytometry, immunocytochemistry, immunohistochemistry, and Western blotting.



Detection of Phospho-ErbB2/Her2 in Human Breast Cancer. Phosphorylated ErbB2/Her2 was detected in immersion-fixed paraffin-embedded sections of human breast cancer tissue using a Rabbit Anti-Human Phospho-ErbB2/Her2 (Y1196) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4438). The tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (Catalog# CTS005; brown) and counterstained with hematoxylin (blue). Specific immunoreactivity was primarily localized to epithelial cells surrounding the mammary ducts.



Detection of Phospho-Akt by Flow Cytometry. Resting Jurkat human acute T cell leukemia cells (open histogram) or Jurkat cells treated with 100 nM Calyculin A (Catalog # 1336) for 30 minutes (filled histogram) were stained with a PE-conjugated Mouse Anti-Human/Mouse Phospho-Akt (S473) Pan Monoclonal Antibody (Catalog # IC7794P).



Detection of Phospho-TOR and Phospho-PRAS40 by Western Blot. (A) Lysates from MCF-7 human breast adenocarcinoma cells were treated with Camptothecin (CPT) (Catalog # 1100). The PVDF membranes were untreated (-) or treated (+) with CIP phosphatase and probed with a Rat Anti-Human Phospho-TOR (S2448) Monoclonal Antibody (Catalog # MAB1665) followed by a HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). **(B)** MCF-7 cells and NIH-3T3 mouse embryonic fibroblast cells were untreated (-) or treated (+) with Recombinant Human IGF-I (Catalog # 291-G1) and PDGF-BB (Catalog # 220-BB), respectively. The PVDF membranes were probed with a Mouse Anti-Human Phospho-PRAS40 (T246) Monoclonal Antibody (Catalog # MAB6890) followed by a HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018).

Akt Pathway Proteins, Antibodies, and ELISAs

INTRACELLULAR AKT REGULATION

Molecule	Proteins	Antibodies	ELISAs
Akt		H (FC, ICC, WB) M (FC, ICC, WB) R (FC, ICC, WB)	
Phospho-Akt (S473)		H (FC, IHC, WB) M (FC, IHC, WB) R (FC, IHC, WB)	H M R
Phospho-Akt (T308)			H M R
Akt1	H	H (FC, ICC, WB) M (WB) R (WB)	H M R
Akt1 (E17K)		H (ICC, WB)	
Phospho-Akt1 (S473)			H M
Phospho-Akt1 (T308)		H (ICC, WB)	
Akt2		H (FC, IHC, WB) M (IHC, WB) R (IHC, WB)	H
Akt3		H (FC, WB)	
GAB2		H (WB)	
GAB3		H (WB)	

Molecule	Proteins	Antibodies	ELISAs
GRB2		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
GRB7		H (WB) M (WB) R (WB)	
PDK-1	H	H (WB) M (WB) R (WB)	
PI 3-Kinase p85α		H (WB) M (WB) R (WB)	
PI 3-Kinase p110β		H (WB)	
PI 3-Kinase p110γ		H (WB)	
PI 3-Kinase p110δ		H (WB)	
PI 3-Kinase p85β		H (WB)	
PTEN	H	H (FC, IHC, WB) M (FC, IHC, WB) R (FC, IHC, WB)	
Phospho-PTEN (S380)		H (WB) M (WB) R (WB)	
Ras		H (WB)	

CELL CYCLE REGULATION

Molecule	Proteins	Antibodies	ELISAs
CDK2		H (WB) M (WB)	
CDK4		H (WB) M (WB)	
E2F-1		H (IHC, WB)	
E2F-2		H (WB)	
E2F-4		H (WB)	
FoxO1/FKHR		H (ICC, WB)	
FoxO3		H (ICC, WB) M (ICC, WB)	
GSK-3α/β		H (FC, ICC, WB) M (FC, ICC, WB) R (FC, ICC, WB)	H M R
Phospho-GSK-3α/β (S21/S9)		H (FC, ICC, WB) M (FC, ICC, WB) R (FC, ICC, WB)	H M R
GSK-3α		H (FC, ICC, IHC, WB) M (FC, ICC, IHC, WB) R (FC, ICC, IHC, WB)	H M R
Phospho-GSK-3α (S21)		H (IHC, WB) M (IHC, WB) R (IHC, WB)	H
GSK-3β	H	H (FC, ICC, WB) M (FC, WB) R (FC, WB)	
Phospho-GSK-3β (S9)		H (FC, ICC, WB)	H M R

Molecule	Proteins	Antibodies	ELISAs
MDM2/HDM2		H (IHC, IP, WB) M (IHC, WB) R (IHC, WB)	H
c-Myc		H (ChIP, FC, ICC, IHC, IP, WB)	
p21/CIP1/CDKN1A		H (FC, IHC, IP, WB)	H
p27/Kip1		H (FC, IHC, WB) M (FC, IHC, WB) R (FC, IHC, WB)	H
Phospho-p27/Kip1 (T157)		H (WB)	
Phospho-p27/Kip1 (T198)		H (WB)	
p53	H	H (ChIP, FC, IP, WB) M (ChIP, IP, WB) R (ChIP, IP, WB)	H M
Phospho-p53 (S15)		H (FC, ICC, IHC, IP, WB)	H
Phospho-p53 (S46)		H (IHC, WB)	H
Phospho-p53 (S392)		H (WB)	H
Phospho-p53 (S18)		M (WB)	
Phospho-p53 (S20)		H (WB)	
Phospho-p53 (S37)		H (WB)	

SPECIES KEY:
H Human, M Mouse, R Rat, B Bovine, Ca Canine, E Equine, F Feline, Ms Multi-species, Zebrafish

APPLICATION KEY:
B/N Blocking/Neutralization, **ChIP** Chromatin Immunoprecipitation, **E** ELISA, **FC** Flow Cytometry, **ICC** Immunocytochemistry, **IF** Immunofluoroscence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **WB** Western blot

Akt Pathway Proteins, Antibodies, and ELISAs (continued)

TOR-REGULATED PROCESSES			
Molecule	Proteins	Antibodies	ELISAs
4EBP1		H (WB) M (WB)	
Beclin 1		H (WB) M (WB) R (WB)	
eIF4B		H (WB)	
eIF4E		H (ICC, WB) M (ICC, WB) R (ICC, WB)	
eIF4G1		H (WB)	
p70 S6 Kinase	H	H (FC, IHC, WB) M (FC, IHC, WB) R (FC, IHC, WB)	H M R
Phospho-p70 S6 Kinase (T421/S424)		H (WB) M (WB) R (WB)	H M R
Phospho-p70 S6 Kinase (T389)		H (WB) M (WB) R (WB)	H M
Phospho-p70 S6 Kinase (T229)		H (ICC, WB) M (WB) R (WB)	
p70 S6 Kinase β		H (WB)	
PDCD4		H (IHC)	
PIK3R4		H (WB)	
Rheb		H (IHC, WB) M (IHC, WB) R (IHC, WB)	
TOR		H (FC, ICC, IP, WB) M (FC, ICC, IP, WB) R (IP, WB)	
Phospho-TOR (S2448)		H (WB)	H
TSC1		H (FC, WB) M (FC, WB) R (FC, WB)	
TSC2		H (ICC, IHC, WB) M (ICC, IHC, WB)	

CELLULAR SURVIVAL			
Molecule	Proteins	Antibodies	ELISAs
APAF-1		H (WB)	
Bad		H (ICC, IHC, WB) M (WB)	H
Phospho-Bad (S112)			H M R
BAK	H	H (WB) M (WB) R (WB)	
Bax		H (IHC, IP, WB) M (IHC, IP, WB) R (IP, WB)	
Bax-α			H
Bcl-x		H (IP, WB) M (IP, WB)	
Bcl-xL	H M	H (IHC, IP, WB) M (IHC, IP, WB) R (IHC, IP, WB)	H M
BIML	H	H (IHC, WB) M (WB)	
Caspase-3	H	H (ICC, IP, WB) M (ICC, IP, WB)	H M
Caspase-9		H (ICC, IHC, WB)	
Cytochrome c	H	H (ICC, IHC, IP, WB) M (IP, WB) R (IP, WB) E (ICC, IHC, IP)	H M R
FoxO1/FKHR		H (ICC, WB)	
FoxO3		H (ICC, WB) M (ICC, WB)	

New products are released weekly.

For the most up-to-date product listing, please visit
www.RnDSystems.com/AktPathway

Akt Pathway Proteome Profiler Arrays

Our membrane-based arrays are ideal for simultaneously determining the expression level and, where indicated, the phosphorylation status of the analytes listed in the table below. These arrays do not require any equipment in addition to what is needed to perform a standard Western blot.

PROTEOME PROFILER MEMBRANE-BASED ARRAYS		
Membrane-based Arrays	Species	Analytes
Phospho-RTK Antibody Array (Catalog # ARY001B)	H	ALK/CD246, Axl, DDR1, DDR2, Dtk, EGF R, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R1, FGF R2α, FGF R3, FGF R4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, Mer, MSP R/Ron, MuSK, PDGF Rα, PDGF Rβ, c-Ret, ROR1, ROR2, Ryk, SCF R/c-kit, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4
Phospho-RTK Antibody Array (Catalog # ARY014)	M	Dtk, EGF R, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphB1, EphB2, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R2 (IIIc), FGF R3, FGF R4, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, Mer, MSP R/Ron, MuSK, PDGF Rα, PDGF Rβ, c-Ret, SCF R/c-kit, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4
Phospho-MAPK Antibody Array (Catalog # ARY002B)	H	Akt1 (S473), Akt2 (S474), Akt3 (S472), Akt pan (S473/S474/S472), CREB (S133), 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), MKK3 (S218/T222), MKK6 (S207/T211), MSK2 (S360), p38α (T180/Y182), p38β (T180/Y182), p38δ (T180/Y182), p38γ (T183/Y185), p53 (S46), p70 S6 Kinase (T421/S424), RSK1 (S380), RSK2 (S386), TOR (S2448)
Phospho-Kinase Antibody Array (Catalog # ARY003B)	H	Akt (S473), Akt (T308), AMPK α1 (T174), AMPK α2 (T172), β-Catenin, Chk-2 (T68), c-Jun (S63), CREB (S133), EGF R (Y1068), eNOS (S1177), ERK1/2 (T202/Y204, T185/Y187), FAK (Y397), Fgr (Y412), Fyn (Y420), GSK-3α/β (S21/S9), Hck (Y411), HSP27 (S78/S82), HSP60, JNK pan (T183/T185, T221/Y223), Lck (Y394), Lyn (Y397), MSK1/2 (S376/S360), p27/Kip1 (T198), p38α (T180/Y182), p53 (S15), p53 (S392), p53 (S46), p70 S6 Kinase (T421/S424), PDGF Rβ (Y751), PLCγ-1 (Y783), PRAS50 (T246), Pyk2 (Y402), RSK1/2/3 (S380), Src (Y419), STAT2 (Y689), STAT3 (S727), STAT3 (Y705), STAT5a (Y699), STAT5a/b (Y699), STAT6 (Y641), TOR (S2448), WNK-1 (T60), Yes (Y426)
Cell Stress Antibody Array (Catalog # ARY018)	H	ADAMTS1, Bcl-2, Carbonic Anhydrase IX, Cited-2, COX-2, Cytochrome c, Dkk-4, FABP1/L-FABP, HIF-1α, HIF-2α, HSP27 (S78/S82), HSP60, HSP70, IDO, JNK pan (T183/Y185), NFκB1, p21/CIP1/CDNK1A, p27/Kip1, p38α (T180/Y182), p53 (S46), PON1, PON2, PON3, Thioredoxin-1, SIRT2, SOD2
Apoptosis Antibody Array (Catalog # ARY009)	H	Bad, Bax, Bcl-2, Bcl-x, Pro-Caspase-3, Cleaved Caspase-3, Catalase, cIAP-1, cIAP-2, Claspin, Clusterin, Cytochrome c, TRAIL R1/DR4, TRAIL R2/DR5, FADD, Fas/TNFSF6, HIF-1α, HO-1/HMOX1/HSP32, HO-2/HMOX2, HSP27, HSP60, HSP70, HTRA2/Omi, Livin, PON2, p21/CIP1/CDNK1A, p27/Kip1, p53 (S15), p53 (S46), p53 (S392), Rad17 (S635), SMAC/Diablo, Survivin, TNF RI/TNFRSF1A, XIAP

Our microplate-based arrays are ideal for simultaneously determining the phosphorylation status of the analytes listed in the table below under multiple experimental conditions. These arrays can also be utilized for high-throughput applications.

PROTEOME PROFILER *96* MICROPLATE-BASED ARRAYS		
Plate-based Arrays*	Species	Analytes
Phospho-RTK Array 1	H	EGF R, ErbB2, ErbB3, ErbB4, HGF R, IGF-I R, Insulin R, M-CSF R, MSP R, PDGF Rα, PDGF Rβ, SCF R, Tie-2, VEGF R1, VEGF R2, VEGF R3
Phospho-RTK Array 2	H	EGF R, EphB4, ErbB2, ErbB3, ErbB4, HGF R, IGF-I R, MSP R
Phospho-RTK Array 3	H	EphB4, PDGF Rα, PDGF Rβ, Tie-1, Tie-2, VEGF R1, VEGF R2, VEGF R3
Phospho-Kinase Array 1	H	Akt (S473), ERK1/ERK2 (T202/Y204), GSK-3β (S9), JNK (T183/Y185), p38α (T180/Y182), p70 S6 Kinase (T421/S424), Src (Y416)

* For a listing of chemiluminescent, near-infrared, and custom array options, please see our website at www.RnDSystems.com/ProteomeProfiler96

SPECIES KEY:
H Human, **M** Mouse, **R** Rat, **B** Bovine, **Ca** Canine, **E** Equine, **F** Feline, **Ms** Multi-species, **Z** Zebrafish

APPLICATION KEY:
B/N Blocking/Neutralization, **ChIP** Chromatin Immunoprecipitation, **E** ELISA, **FC** Flow Cytometry, **ICC** Immunocytochemistry, **IF** Immunofluoroscence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **WB** Western blot

Proteins, Antibodies, and ELISAs for Akt Pathway Activation

GROWTH FACTORS FOR RTK DEPENDENT AKT ACTIVATION

Molecule	Proteins	Antibodies	ELISAs
Amphiregulin	H M	H (B/N, E, ICC, IHC, WB) M (B/N, E, IHC, WB)	H M
Angiopoietin-1	H	H (E, IHC, WB)	H
Angiopoietin-2	H M	H (E, IHC, WB) M (WB)	H
Angiopoietin-3	M	M (E, IHC, WB)	M
Angiopoietin-4	H	H (B/N, E, IHC, WB)	H
BDNF	H	H (E, FC, IHC, WB)	H
Betacellulin/BTC	H M	H (B/N, E, IHC, WB) M (B/N, E, IHC, WB)	H M
EGF	H M R	H (B/N, E, IHC, WB) M (B/N, E, IHC, WB) R (B/N, E, WB)	H M R
EGF-L6	M		
Epiregulin	H M	H (B/N, FC, IHC, WB) M (B/N, E, WB)	M
FGF acidic	H M B	H (B/N, IHC, WB) M (B/N, WB) B (B/N, WB)	H
FGF basic	H M R B	H (B/N, E, IHC, WB) B (B/N, WB)	H
FGF-3	H	H (B/N, IHC, WB)	
FGF-4	H M	H (B/N, E, IHC, WB) M (IHC, WB)	H
FGF-5	H	H (B/N, IHC, WB)	
FGF-6	H M	H (B/N, E, WB) M (IHC)	
KGF/FGF-7	H M Ca	H (B/N, E, IHC, WB) Ca (ICC, IHC)	H
FGF-8	H M	H (B/N, IHC, WB) M (B/N, IHC, WB)	
FGF-10	H M	H (IHC, WB) M (IHC, WB)	
FGF-13		H (WB)	
FGF-15		M (IHC)	
FGF-17	H M	H (B/N, IHC, WB)	
FGF-19	H	H (B/N, E, IHC, WB)	H
FGF-20	H	H (B/N, ICC, WB)	
FGF-21	H	H (FC, ICC, WB) M (WB)	H M R
FGF-23	H M	H (B/N, WB) M (IHC, WB)	
HB-EGF	H	H (B/N, E, FC, IHC, WB)	H
IGF-I	H M R	H (B/N, E, ICC, IHC, WB) M (B/N, E, IHC, WB)	H M R

Molecule	Proteins	Antibodies	ELISAs
IGF-II	H M	H (B/N, IHC, WB) M (B/N, E, IHC, WB)	M
Insulin		H (FC, ICC, IHC) M (FC, ICC) B (FC, ICC)	
Proinsulin	H	H (E, FC, ICC) M (FC, ICC)	H
LRIG1	M	H (FC, IHC) M (B/N, FC, ICC, WB)	
NRG1	H	H (B/N, E, IHC, WB)	H
NRG1-α/HRG1-α	H	H (B/N, IHC, WB)	
NRG1-β1/HRG1-β1	H	H (B/N, E, IHC, WB)	H
β-NGF	H M R	H (B/N, E, IHC, WB) R (B/N, E, IHC, WB)	H R
NT-3	H	H (B/N, E, IHC, WB)	H
NT-4	H M	H (B/N, E, IHC, WB)	H
PDGF	H P	H (B/N, WB) Ms (B/N, WB)	
PDGF-AA	H R	H (B/N, E, IHC, WB) R (B/N, IHC, WB) Ms (B/N, WB)	H M
PDGF-AB	H R	H (B/N, E, IHC, WB) Ms (B/N, WB)	H M R
PDGF-BB	H R	H (B/N, E, WB) Ms (B/N, WB)	H M R
PDGF-CC	H M	H (B/N, IHC, WB) M (B/N, IHC, WB)	H
PDGF-DD	H	H (B/N, IHC, WB)	H
PlGF	H	H (E, IHC, WB)	H
PlGF-2	H M	M (B/N, E, WB)	M
TGF-α	H	H (B/N, E, IHC, WB)	H
VEGF*	H M R Ca F Z	H (B/N, E, FC, ICC, IF, IHC, WB) M (B/N, E, IHC, WB) R (B/N, E, IHC, WB) Ca (B/N, E, ICC, WB) Z (B/N, WB)	H M R Ca
VEGF/PlGF Heterodimer	H	H (WB)	H
VEGF-B	H M	H (IHC, WB) M (B/N, IHC, WB)	
VEGF-C	H	H (IHC, WB) M (WB) R (WB)	H
VEGF-D	H M	H (B/N, E, IHC, WB) M (E, IHC, WB)	H M

*Please visit our website to view our isoform-specific VEGF products, including VEGF 165, VEGF 165b, VEGF 162, VEGF 145, VEGF 121, and VEGF 111.

RTKS THAT ACTIVATE AKT

Molecule	Proteins	Antibodies	ELISAs
EGF R/ErbB1	H M	H (E, FC, ICC, IHC, IP, WB) M (FC, IHC, WB)	H
Phospho-EGF R/ErbB1			H
Phospho-EGF R/ErbB1 (Y1068)		H (FC, WB)	H
Phospho-EGF R/ErbB1 (Y1173)		H (FC, ICC, IHC, WB)	
Phospho-EGF R/ErbB1 (Y845)		H (FC, ICC, IHC, WB)	
ErbB2/Her2	H	H (B/N, E, FC, ICC, IHC, WB) M (FC, IHC, WB)	H
Phospho-ErbB2/Her2			H
Phospho-ErbB2/Her2 (Y1196)		H (IHC, WB)	H
Phospho-ErbB2/Her2 (Y1248)		H (IHC, WB)	
ErbB3/Her3	H M	H (B/N, E, FC, IHC, WB) M (FC, IHC, WB)	H
Phospho-ErbB3/Her3			H
Phospho-ErbB3/Her3 (Y1262)		H (WB)	H
ErbB4/Her4	H M	H (FC, WB)	H
Phospho-ErbB4/Her4			H
Phospho-ErbB4/Her4 (Y1188)			H
Phospho FGF R1-4 (Y653/Y654)		H (ICC, WB)	
FGF R1	H	H (B/N, WB)	
Phospho-FGF R1			H
FGF R1α	H		
FGF R1β	H		
FGF R2	H M	H (B/N, FC, ICC, IHC, WB) M (B/N, WB)	H
Phospho-FGF R2			H
FGF R2α	H M	H (WB)	H
Phospho-FGF R2α			H
FGF R2β	H M		
FGF R3	H M	H (B/N, FC, ICC, IHC, WB) M (B/N)	H
Phospho-FGF R3			H
FGF R4	H M	H (FC, ICC, IHC, WB) M (IHC, WB)	H
Phospho-FGF R4			H
HGF R/c-MET	H M Ca	H (B/N, E, FC, IHC, WB) M (B/N, E, IHC, WB) Ca (B/N, E, WB)	H M Ca
Phospho-HGF R/c-MET			H
Phospho-HGF R/c-MET (Y1234/Y1235)		H (FC, IHC, WB) M (FC, IHC, WB)	H
Phospho-HGF R/c-MET (Y1349)		H (IHC, WB) M (IHC, WB)	
Phospho-HGF R/c-MET (Y1003)		H (ICC, IHC, WB)	
IGF-I R	H M	H (B/N, E, FC, IHC, WB) M (B/N, IHC, WB)	H

Molecule	Proteins	Antibodies	ELISAs
Phospho-IGF-I R			H
Phospho-INS R (Y1162/3)/IGF-I R (Y1135/6)		H (FC, ICC, WB)	
IGF-II R	H	H (B/N, E, FC, IHC, WB)	H
Insulin R/CD220	H M	H (FC, ICC, IHC, WB) M (FC)	H
Phospho-Insulin R/CD220			H
INSRR		H (FC, IHC, WB)	
PDGF Rα	H M	H (B/N, FC, IHC, IP, WB) M (B/N, IHC, WB)	H
Phospho-PDGF Rα			H
Phospho-PDGF Rα (Y742)		H (IHC, WB)	H M
PDGF Rβ	H M	H (B/N, FC, IHC, IP, WB) M (IHC, WB)	H
Phospho-PDGF Rβ			H
Phospho-PDGF Rβ (Y751)		H (WB)	H
Phospho-PDGF Rβ (Y1021)		H (WB)	H M
Tie-1	H	H (FC, ICC, IHC, WB)	H
Tie-2	H M R Z	H (B/N, E, FC, IHC, WB) M (B/N, WB) R (B/N, WB) Z (B/N, IHC, WB)	H M
Phospho-Tie-2			H M
Phospho-Tie-2 (Y992)		H (FC, WB) M (FC, WB)	H
Phospho-Tie-2 (Y1102/Y1100)		H (IHC, WB) M (IHC, WB)	
TrkA	H R	H (B/N, FC, IHC, WB) R (IHC, WB)	H
Phospho-TrkA			H
Phospho-TrkA (Y785)		H (WB) R (WB)	R
TrkB	H M	H (FC, IHC, WB) M (B/N, IHC, WB)	H
Phospho-TrkB			H
TrkC	H M	H (B/N, FC, IHC, WB) M (B/N, IHC, WB)	H
Phospho-TrkC			H
VEGF R1/Flt-1	H M	H (B/N, E, FC, IHC, WB) M (B/N, E, FC, IHC, WB)	H M
Phospho-VEGF R1/Flt-1			H
Phospho-VEGF R1/Flt-1 (Y1213)		H (WB)	
VEGF R2/KDR/Flk-1	H M	H (B/N, E, FC, ICC, IHC, WB) M (B/N, E, FC, ICC, IHC, WB)	H M
Phospho-VEGF R2/KDR/Flk-1			H
Phospho-VEGF R2/KDR/Flk-1 (Y1214)		H (FC, ICC, WB)	
VEGF R3/Flt-4	H M	H (E, FC, ICC, IHC, WB) M (E, FC, WB)	H M
Phospho-VEGF R3/Flt-4			H

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Manipulate PI 3-Kinase/Akt Signaling with Tocris Inhibitors

Tocris Biosciences, an R&D Systems company, offers many inhibitors for the modulation of intracellular signaling. The table below lists inhibitors that target molecules involved in PI 3-Kinase/Akt signaling along with the model system and dose used in the study referenced. For more information about these and other inhibitors, please visit the Tocris Biosciences website at **www.tocris.com**.

PI 3-KINASE/AKT PATHWAY INHIBITORS

Target	Inhibitor	Catalog #	Model System	Effective Dose*	Reference
Akt	API-2	2151	Cell Culture	1 µM	Wu, Y. *et al.* (2010) Cancer Res. **70**:5475.
Akt	API-2	2151	Mouse	5 µg	Noh, K.H. *et al.* (2009) Mol. Ther. **17**:439.
Akt	SC 66	4398	Cell Culture	15 µM	Jo, H. *et al.* (2011) Proc. Natl. Acad. Sci. USA **108**:6486.
Akt	SC 66	4398	Mouse	15 & 30 mg/kg	Jo, H. *et al.* (2011) Proc. Natl. Acad. Sci. USA **108**:6486.
Akt	GSK 690693	4144	Cell Culture	20 µM	Makhov, P.B. *et al.* (2012) Mol. Cancer Ther. **11**:1510.
Akt	GSK 690693	4144	Mouse	30 mg/kg	Carol, H. *et al.* (2010) Pediatr. Blood Cancer **55**:1329.
CDK4	Arcyriaflavin A	2457	Cell Culture	20 µM	Robey, R.W. *et al.* (2007) Mol. Cancer Ther. **6**:1877.
CDK4	NSC 625987	2152	*In vitro*	10 µM	Kubo, A. *et al.* (1999) Clin. Cancer Res. **5**:4279.
GSK-3	SB 216763	1616	Cell Culture	5 µM	Spokoini, R. *et al.* (2010) Mol. Endocrinol. **24**:1136.
GSK-3	SB 216763	1616	Mouse	10 mg/kg	Kapfhamer, D. *et al.* (2010) J. Neurosci. **30**:8830.
GSK-3	BIO	3194	Cell Culture	1 µM	Cao, H. *et al.* (2012) PLoS One **7**:e31502.
GSK-3	BIO	3194	Mouse	250 µg/kg	Trivedi, C.M. *et al.* (2007) Nat. Med. **13**:324.
GSK-3	SB 415286	1617	Cell Culture	7.5 µM	Alabed, Y.Z. *et al.* (2010) J. Neurosci. **30**:5635.
MDM2	Nutlin-3	3984	Cell Culture	7 µM	Santag, S. *et al.* (2012) Oncogene [Epub ahead of print].
MDM2	Nutlin-3	3984	Mouse	40 mg/kg	Endo, S. *et al.* (2011) Cancer Sci. **102**:605.
MDM2	RITA	2443	Cell Culture	1-10 µM	Saha, M.N. *et al.* (2010) Mol. Cancer Ther. **9**:3041.
MDM2	RITA	2443	Mouse	1 & 10 mg/kg	Issaeva, N. *et al.* (2004) Nat. Med. **10**:1321.
p70 S6 Kinase	PF 4708671	4032	Cell Culture	10 µM	Pearce, L.R. *et al.* (2010) Biochem. J. **431**:245.
p70 S6 Kinase	PF 4708671	4032	Mouse	75 mg/kg	Di, R. *et al.* (2012) Biochem. J. **441**:199.
PDK-1	GSK 2334470	4143	Cell Culture	0.3-3 µM	Najafov, A. *et al.* (2011) Biochem. J. **433**:357.
PI 3-Kinase	LY 294002	1130	Cell Culture	25 µM	Qin, J. *et al.* (2012) J. Biol. Chem. **287**:13620.
PI 3-Kinase	LY 294002	1130	Mouse	50 mg/kg	Brown, J.B. *et al.* (2011) Infect. Immun. **79**:1863.
PI 3-Kinase	Wortmannin	1232	Cell Culture	5 µM	Qin, J. *et al.* (2012) J. Biol. Chem. **287**:13620.
PI 3-Kinase	Wortmannin	1232	Mouse	100 mg/kg	Brown, J.B. *et al.* (2011) Infect. Immun. **79**:1863.
TOR	KU 0063794	3725	Cell Culture	2 µM	Case, N. *et al.* (2011) J. Biol. Chem. **286**:39450.
TOR	PP 242	4257	Cell Culture	400 nM	Janes, M.R. *et al.* (2010) Nat. Med. **16**:205.
TOR	PP 242	4257	Mouse	60 mg/kg	Dormond-Meuwly, A. *et al.* (2011) Biochem. Biophys. Res. Commun. **407**:714.
TOR	Rapamycin	1292	Cell Culture	1-100 nM	Kobayashi, S. *et al.* (2007) Cancer Sci. **98**:726.
TOR	Rapamycin	1292	Mouse	5 mg/kg	Wahdan-Alaswad, R.S. *et al.* (2012) Mol. Cancer Res. **10**:821.
TOR	Torin 1	4247	Cell Culture	250 nM	Peterson, T.R. *et al.* (2011) Cell **146**:408.
TOR	Torin 2	4248	Cell Culture	1.5 µM	Settembre, C. *et al.* (2012) EMBO J. **31**:1095.
TOR	Torin 2	4248	Mouse	20 mg/kg	Liu, Q. *et al.* (2012) J. Med. Chem. **55**:250.

**Doses listed are intended for general reference only. The most effective dose to use will need to be determined for the specific conditions of each experiment.*