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Akt/PI 3-Kinase Signaling in Cell Death and Cell Survival

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Research Tools for Akt/PI 3-Kinase Signaling

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Akt/PI 3-Kinase Signaling in Cell Death and Cell Survival

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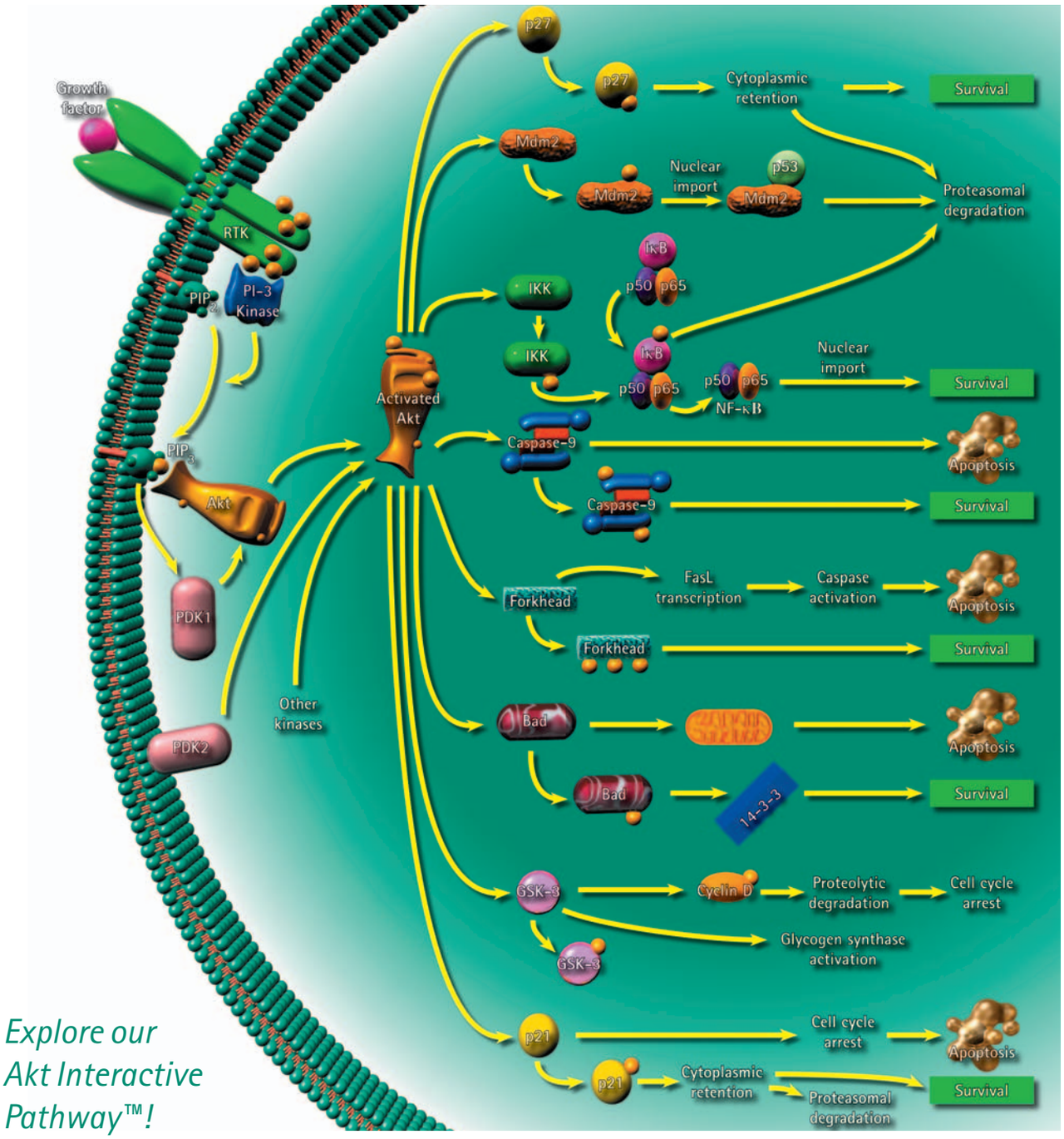
Akt (protein kinase B), a serine/threonine kinase, has emerged as a critical enzyme in signal transduction pathways involved in cell proliferation, apoptosis, angiogenesis, and diabetes. In mammals three isoforms of Akt (α, β, γ or Akt 1, 2, 3) are reported that exhibit a high degree of homology, but differ slightly in the localization of their regulatory phosphorylation sites. Aktα is the predominant isoform in most tissues, whereas the highest expression of Aktβ is observed in insulin-responsive tissues, and Aktγ is abundant in brain tissue. Each Akt isoform is composed of three functionally distinct regions: an N-terminal pleckstrin homology (PH) domain that provides a lipid-binding module to direct Akt to PIP_2 and PIP_3, a central catalytic domain, and a C-terminal hydrophobic motif.

Akt is constitutively phosphorylated at Ser^{124}, in the region between the PH and catalytic domains, and on Thr^{450}, in the C-terminal region (in Aktα, the most widely studied isoform) in unstimulated cells. Activation of Akt involves growth factor binding to a receptor tyrosine kinase and activation of PI 3-K, which phosphorylates membrane bound PIP_2 to generate PIP_3. The binding of PIP_3 to the PH domain anchors Akt to the plasma membrane and allows its phosphorylation and activation by PDK1. Akt is fully activated following its phosphorylation at two regulatory residues, a threonine residue on the kinase domain and a serine residue on the hydrophobic motif, which are structurally and functionally conserved within the AGC kinase family. Phosphorylation at Thr^{308} and Ser^{473} is required for the activation of Aktα, while phosphorylation at Thr^{309} and Ser^{474} activates Aktβ. Phosphorylation at Thr^{305} activates Aktγ. Phosphorylation of a threonine residue on the kinase domain, catalyzed by PDK1, is essential for Akt activation. It causes a charge-induced conformational change, allowing substrate binding and increased rate of catalysis. Akt activity is augmented about 10-fold by phosphorylation at the serine residue by PDK2. DNA-PK and $PKC_{\beta II}$ are reported to phosphorylate the serine residue on the regulatory subunit. Without threonine phosphorylation, the hydrophobic motif of Akt is more susceptible to the action of phosphatases; however, the dually phosphorylated and fully active enzyme is stable, allowing its localization to the nucleus and other sites. The activity of Akt is negatively regulated by PTEN and SHIP.

The principal role of Akt is to facilitate growth factor-mediated cell survival and to block apoptotic cell death. This is achieved by phosphorylating and deactivating pro-apoptotic factors such as Bad, caspase-9, and Forkhead transcription factors (AFX, Daf-16, FKHR). The phosphorylation of Bad at Ser^{136} promotes its association with 14-3-3 proteins in the cytosol, which prevents Bad from localizing at the mitochondria to induce apoptosis. Akt is also known to promote cell survival by inactivating caspase-9 through phosphorylating it at Ser^{196}. Likewise, activated Akt phosphorylates Forkhead family members, resulting in their sequestration in the cytoplasm. In the absence of survival factors and Akt activity, Forkhead family members translocate to the nucleus, where they initiate a program of gene expression (e.g., FasL) that promotes cell death. Akt is also reported to phosphorylate IKKα at Thr^{23} and activate it. The activated IKKα, in turn, phosphorylates IκB, targeting it for ubiquitination and proteasomal degradation. This leads to the activation and nuclear translocation of NF-κB, and transcription of NF-κB-dependent pro-survival genes, including Bcl-x_L and caspase inhibitors. Akt also phosphorylates and inactivates GSK-3, allowing the activation of glycogen synthase to proceed. An important point to note is that phosphorylation of cyclin D by GSK-3 targets it for proteolysis; hence the inactivation of GSK-3 may promote the up-regulation of cyclin D and enhance cell cycling. Recently it has been shown that when Chk1, a DNA damage effector kinase, is phosphorylated by Akt at Ser^{280} it can no longer be phosphorylated by ATM/ATR at Ser^{345} to undergo activation. This may be of therapeutic significance as Chk1 inhibition is shown to enhance sensitization of tumors to



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chemotherapeutic agents. Akt also phosphorylates Cdc25B on Ser^{353}, resulting in its cytoplasmic accumulation. Cdc25B undergoes activation during S-phase and plays a role in activating the mitotic kinase Cdk1/cyclin B in the cytoplasm. In relocating Cdc25B to the cytoplasm, Akt regulates its function and participates in controlling the entry of cells into mitosis.

A number of oncogenes and tumor suppressor genes that function upstream of Akt influence cancer progression by regulating Akt. Aktα is expressed to various degrees in breast cancer cell lines and is important in estrogen-stimulated growth. Treatment of multiple myeloma cell lines with the Akt inhibitor, 1L-6-Hydroxymethyl-*chiro*-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate (Cat. No. 124005), results in reduced survival of both drug resistant and drug sensitive cells. Akt plays a critical role in tumorigenesis, becoming activated when tumor suppressors such as p27 and PTEN lose

their functions. Phosphorylation of p27 at Thr^{157} by Akt impairs its nuclear import. Cytoplasmic mislocalization of p27 has been strongly linked to loss of differentiation and poor outcome in breast cancer. Akt is also reported to physically associate with endogenous p21, a cell cycle inhibitor, and phosphorylate it at Thr^{145}, causing its localization to the cytoplasm and subsequent degradation.

Akt and p53 play opposing roles in signaling pathways that determine cell survival and the interaction between these two molecules is becoming an important area of study. Under conditions where the apoptotic effect of p53 is dominant, destruction of Akt plays a role in accelerating the apoptotic process. In apoptosis-prone cells, p53-dependent signaling enables downregulation of Akt, which predisposes cells to rapid apoptosis in response to stress signals. Under certain circumstances Akt activation may overcome the death promoting effects of p53 and may rescue cells from apoptosis. It has been reported that Akt can phosphorylate Mdm2 on Ser^{166} and Ser^{188} and promote its translocation to the nucleus where it destabilizes p53 and enhances its degradation via the proteasomal pathway.

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Products for Akt/Protein Kinase B Related Research

Akt Antibodies

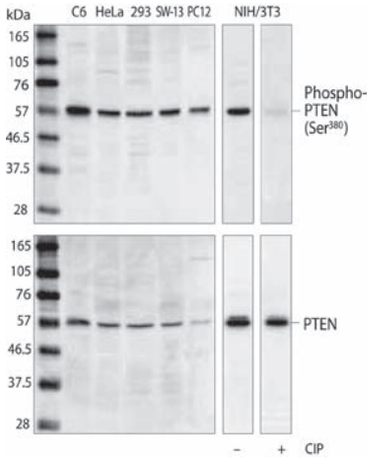
Product	Cat. No.	Comments	Size
Anti-Akt1 (Ab-1) (135-145) Rabbit pAb	PC510	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 135-145 of human Akt1. Reacts with human and mouse. **IF**	50 µl
Anti-Akt1 (88-100) Rabbit pAb	530311	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acids residues 88-100 (Cat. No. 530312) of Akt1. Detects the ~60 kDa Akt in a variety of rat and mouse tissues and human cell lines. **ELISA, IB, IP**	100 µg
Anti-Akt PH Domain Mouse mAb (SKB1)	ST1088	Monoclonal IgG, protein G-purified. Immunogen used was a GST-fusion protein corresponding to residues 1-149 of human Akt 1. Detects the ~60 kDa Akt in human and rat. **FC, IB, IP**	50 µg
PhosphoDetect™ Anti-Akt1 ($pSer^{473}$) Mouse mAb (IIE6)	124003	Monoclonal IgG_1, immunoaffinity-purified. Clone 11E6. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding the Ser^{473} of human Akt1. Recognizes the ~60 kDa Akt1 phosphorylated at Ser^{473} in human and mouse. **ELISA, IB**	1 set
PhosphoDetect™ Anti-Akt1 ($pThr^{308}$) Rabbit pAb	124001	Polyclonal IgG, purified by thiophilic adsorption and size exclusion chromatography. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding the Thr^{308} of human Akt1. Recognizes the ~60 kDa human and mouse Akt1 phosphorylated at Thr^{308}. Set includes a vanadate-treated 224 HepG2 positive control. **FC, IB**	10T
Anti-Akt2 (Ab-1) (108-121) Rabbit pAb	PC511	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 108-121 of human Akt2. Reacts with human and mouse. **IF**	50 µl
Anti-Akt2 Rabbit pAb	124002	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to a 16-amino acid sequence at the C-terminus of Akt2. Reacts with human, mouse, and rat. **ELISA, IB**	100 µl
Anti-Akt3 (Ab-1) (130-143) Rabbit pAb	PC512	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 130-143 of human and mouse Akt3 protein. **IF**	50 µl
Anti-Akt3 Rabbit pAb	124004	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to a 12-amino acid sequence at the C-terminus of Akt3. Reacts with human, mouse, and rat. **ELISA, IB**	100 µl

ELISA: enzyme-linked immunosorbent assay; **FC**: flow cytometry; **IB**: immunoblotting; **IF**: immunofluorescence; **IP**: immunoprecipitation; **mAb**: monoclonal antibody; **pAb**: polyclonal antibody; **10T**: 10 tests by Western miniblots

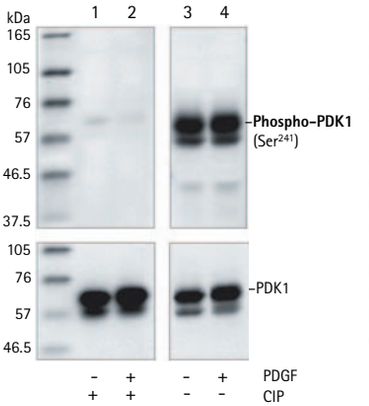
Other Akt-Related Antibodies

Product	Cat. No.	Comments	Size
Anti-PDK1 (285-559) Rabbit pAb	ST1036	Polyclonal, undiluted serum. Immunogen used was C-terminus of mouse PDK1 (amino acid residues 285-559) fused to GST. Antibody detects the ~64 kDa PDK1 in hamster, human, and mouse. **IB, IP**	50 µl
Anti-PI 3-Kinase p110δ, C-Terminal (1026-1044) Rabbit pAb	526553	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide [(C)SWKTKVNWLAHNVSKDNRQ] corresponding to a distinct C-terminal region of the human phosphatidylinositol 3-kinase p110δ. **IB, IC**	100 µl
PhosphoDetect™ Anti-PDK1 (pSer^{241}) Rabbit pAb	ST1073	Polyclonal IgG, protein A and peptide affinity purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser^{241} of PDK1. Detects the ~63 kDa PDK1 phosphorylated on Ser^{241} in human, mouse, and rat. **IB, IC, IP**	50 µl
PhosphoDetect™ Anti-PRAS40 (pThr^{246}) Rabbit pAb	PS1011	Polyclonal IgG, immunoaffinity-purified. Immunogen was a synthetic phosphopeptide corresponding to amino acid residues surrounding Thr^{246} of human PRAS40. Recognizes the ~40 kDa PRAS40 phosphorylated at Thr^{246} in human and mouse. **IB**	10T
PhosphoDetect™ Anti-PTEN (pSer^{380}) Rabbit pAb	ST1072	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser^{380} of PTEN. Detects the ~54 kDa PTEN phosphorylated on Ser^{380} in human, mouse, and rat. **IB, IC, IP, PS**	50 µl

IB: immunoblotting; **IC**: immunocytochemistry; **IF**: immunofluorescence; **IP**: immunoprecipitation; **PS**: paraffin sections; **10T**: 10 tests by Western miniblots



Detection of human PTEN phosphorylated on Ser^{380} by immunoblotting. Lysates from C6, HeLa, 293, SW-13, PC-12 (all untreated) and NIH-3T3 cells untreated or treated with calf intestinal alkaline phosphatase (CIP). Primary antibody Phospho-Detect™ Anti-PTEN (pSer^{380}), Rabbit pAb (Cat. No. ST1072, top panel).



Detection of human PDK1 phosphorylated on Ser^{241} by immunoblotting.
Samples: Lysates from NIH-3T3 cells (serum starved for 16 hours), treated with calf intestinal alkaline phosphatase (CIP) (lanes 1 and 2); untreated (lane 3) or treated with 50 ng/ml platelet derived growth factor (PDGF) (lanes 2 and 4).
Primary antibody: Phospho-Detect™ Anti-PDK1, (pSer^{241}), Rabbit pAb (Cat. No. ST1073) or Anti-PDK1 (bottom panel).

Recombinant Akt and Akt Substrates

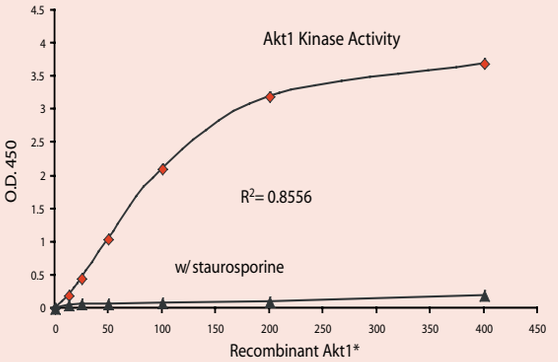
Product	Cat. No.	Comments	Size
Akt1, His•Tag®, Activated, Human, Recombinant, *S. frugiperda*	124006	A purified recombinant human Akt1 expressed in *Spodoptera frugiperda* cells. Highly active form of Akt1 suitable for labeling Akt substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Specific activity: ≥2700 units/mg protein. *Purity:≥95% by SDS-PAGE.* M.W. 60,000.	20 µg
AKTide-2T (ARKRERTYSFGHHA)	123900	An optimal peptide substrate for assaying Akt/PKB/Rac-protein kinase activity *in vitro*. The peptide undergoes phosphorylation at the Ser site (K_m = 3.9 µM). Competitively inhibits histone H2B phosphorylation by Akt (K_i = 12 µM). *Purity:≥95% by HPLC.*	1 mg
AKTide-SA (ARKRERAYAFGHHA)	123905	Serves as a negative control for AKTide-2T (Cat. No. 123900). Lacks the Ser phosphorylation site.*Purity:≥95% by HPLC.*	1 mg

Akt Assay Kits

Product	Cat.No.	Comments	Size
Akt Activity Immunoassay Kit	124007	A non-radioactive assay kit for measuring Akt activity in cell lysates or tissue extracts. Akt is first enriched via immunoprecipitation with an anti-Akt antibody and then tested for its ability to phosphorylate GSK-3α, an Akt substrate. Phosphorylated GSK-3α is detected through immunoblotting with anti-GSK-3α phospho-specific antibody.	1 kit
Akt, Phospho-Specific (Thr^{308}) ELISA Kit	CBA004	A solid-phase sandwich ELISA kit that employs a monoclonal antibody specific for Akt (regardless of phosphorylation state) coated onto the wells of a 96-well plate. Detects Akt phosphorylated on Thr^{308}. The sensitivity of this ELISA was compared to Western blotting using known quantities of Akt ($pThr^{308}$). Although this kit was developed for human samples, it has also been found to cross-react with mouse and rat.	1 kit
Akt, Phospho-Specific (Ser^{473}) ELISA Kit	CBA005	A solid-phase sandwich ELISA kit that employs a monoclonal antibody specific for Akt (regardless of phosphorylation state) coated onto the wells of a 96-well plate. This kit is designed to detect and quantify the level of Akt protein that is phosphorylated at Ser^{473}. Although designed for use with human cell lines, cross-reactivity with mouse and rat cells has also been observed.	1 kit

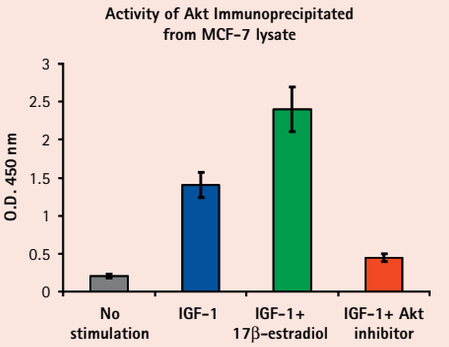
K-LISA™ Akt Activity Kit

This 96-well ELISA-based kit is designed for the colorimetric detection of Akt activity in purified or partially purified preparations and for *in vitro* Akt inhibitor screening. The kit utilizes an N-terminal biotinylated peptide substrate (GRPRTSSFAEG) that is phosphorylated on the second serine by Akt1, Akt2, and Akt3.



(*580 Units/mg; 1 Unit is equal to 1 nmol phosphate incorporated in substrate per min at 30°C)

Activity of purified Akt in the presence and absence of staurosporine (Cat. No. 569396). The activity of recombinant human Akt1 (Cat. No. 124006) (15-400 ng) was determined using the K-LISA™ Akt Activity Kit. Final concentration of staurosporine was 1 µM. Assay range: 10-200 ng (580 units/mg).



Activity of Akt immunoprecipitated from MCF-7 cell lysates. Near-confluent MCF-7 cells were stimulated with IGF-1 (100 ng/ml) or IGF-1 (100 ng/ml) and 17β-estradiol (Cat. No. 3301) (500 nM) for 30 min at 37°C. For inhibition of Akt, cells were pre-incubated at 37°C for 15 min in the presence of Akt Inhibitor II (Cat. No. 124008) followed by stimulation with IGF-1 (100 ng/ml) for 30 min at 37°C. Cell lysates were prepared using PhosphoSafe™ Extraction Reagent (Cat. No. 71296-3). Equal amounts of total protein (1.5 mg) were immunoprecipitated and activity was determined.

Cat. No. CBA019 1 kit

Technical Tips for use of Akt inhibitors:

Our Akt inhibitors are classified into four groups based on their mode of actions.

The first three groups of inhibitors interfere with the cellular activation of Akt and do not affect already activated Akt. These inhibitors should only be used on cells (with the exception of Cat. No. 124013, which is not cell-permeable) or in coupled kinase assays, involving non-activated Akt. The fourth group of inhibitors is suitable for use in cell cultures, as well as in cell-free kinase assays, involving either activated or non-activated Akts.

Group I: Phosphatidylinositol analogs (Cat. Nos. 124005/124008/124009)
These inhibitors compete with PIP_2 thereby preventing the generation of PIP_3 . They also compete with PIP_3 binding to Akt. The phosphonate analogs (124008/124009) display improved metabolic stability over the carbonate analog (124005).

Group II: (Cat. Nos. 124011/124012/124015)
These inhibitors target yet to be identified signaling molecules, other than PDK1 and PI 3-K.

Group III: (Cat. Nos. 124013/124014)
These inibitors contain a TCL1-derived peptide inhibitor sequence, which binds to the PH domain of Akt and interferes with the Akt-phosphoinositide interaction.

Group IV: (Cat. No. 124018)
This inhibition is PH domain-dependent. Inhibition is not seen in Akts lacking the PH domain or closely related AGC family kinases. This inhibitor has the distinct advantage of directly binding to either non-activated or activated Akt, thereby inhibiting both the activation of Akt and the kinase activity of Akt.

Akt/PI 3-Kinase Inhibitors

Product	Cat. No.	Enzyme Targets	Cell Permeable	Comments	Size
Akt Inhibitor	124005	Akt, PI 3-K	Yes	PI analog, prevents PIP_3 formation and binding to Akt (IC_{50} = 5.0 µM). PI 3-K (IC_{50} = 83 µM)	1 mg
Akt Inhibitor II (SH-5)	124008	Akt, PI 3-K	Yes	PI analog, prevents PIP_3 formation and binding to Akt. Metabolically more stable than Akt Inhibitor (Cat. No. 124005).	1 mg
Akt Inhibitor III (SH-6)	124009	Akt, PI 3-K	Yes	PI analog, prevents PIP_3 formation and binding to Akt. Metabolically more stable than Akt Inhibitor (Cat. No. 124005).	1 mg
Akt Inhibitor IV	124011	unknown	Yes	ATP-competitive inhibitor of a kinase upstream of Akt but downstream of PI 3-K.	1 mg 5 mg
InSolution™Akt Inhibitor IV	124015	unknown	Yes	Supplied as a 10 mM solution of Akt Inhibitor IV (Cat. No. 124011) in DMSO.	1 mg
Akt Inhibitor V, Triciribine (API-2, NSC 154020, TCN)	124012	unknown	Yes	Inhibits the cellular phosphorylation/activation of Akt1/2/3 by targeting an Akt effector molecule other than PI 3-K or PDK1. Has shown efficacy *in vivo*.	1 mg
Akt Inhibitor VI, *Akt-in*	124013	Akt	No	A15-mer peptide that directly binds Akt PH domain (k_d=18µM), preventing PI binding.	2 mg
Akt Inhibitor VII, *TAT-Akt-in*	124014	Akt	Yes	A cell-permeable version of Cat. No. 124013 that directly binds Akt PH domain, preventing PI binding. Has shown efficacy *in vivo*.	2 mg
Akt Inhibitor VIII, Isozyme-Selective, Akt-1/2	124018	Akt	Yes	Inhibition is PH domain dependent. Shown to block basal and stimulated phosphorylation/activation of Akt1/Akt2 in cultured cells *in vitro* and in mice *in vivo* (IC_{50} = 58 nM, 210 nM, and 2.12 µM for Akt1, Akt2, and Akt3, respectively, in *in vitro* kinase assays).	1 mg
LY 294002	440202	PI 3-K	Yes	A potent, and specific inhibitor of PI 3-K (IC_{50} = 1.4 µM). Acts on the ATP-binding site of the enzyme.	5 mg
InSolution™ LY 294002	440204	PI 3-K	Yes	Supplied as a 10 mM (1 mg/325 µl) solution of LY 294002 (Cat. No. 440202) in DMSO.	1 mg
Quercetin, Dihydrate	551600	PI 3-K, PLA_2	No	An inhibitor of PI 3-kinase (IC_{50} = 3.8 µM) and phospholipase A_2 (IC_{50} = 2 µM).	100 mg
Wortmannin	681675	PI 3-K	Yes	A potent, selective, and irreversible inhibitor of PI 3-K (IC_{50} = 5 nM). Blocks the catalytic activity of PI 3-kinase without affecting the upstream signaling events.	1 mg

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