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

Second Edition

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What's Inside

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

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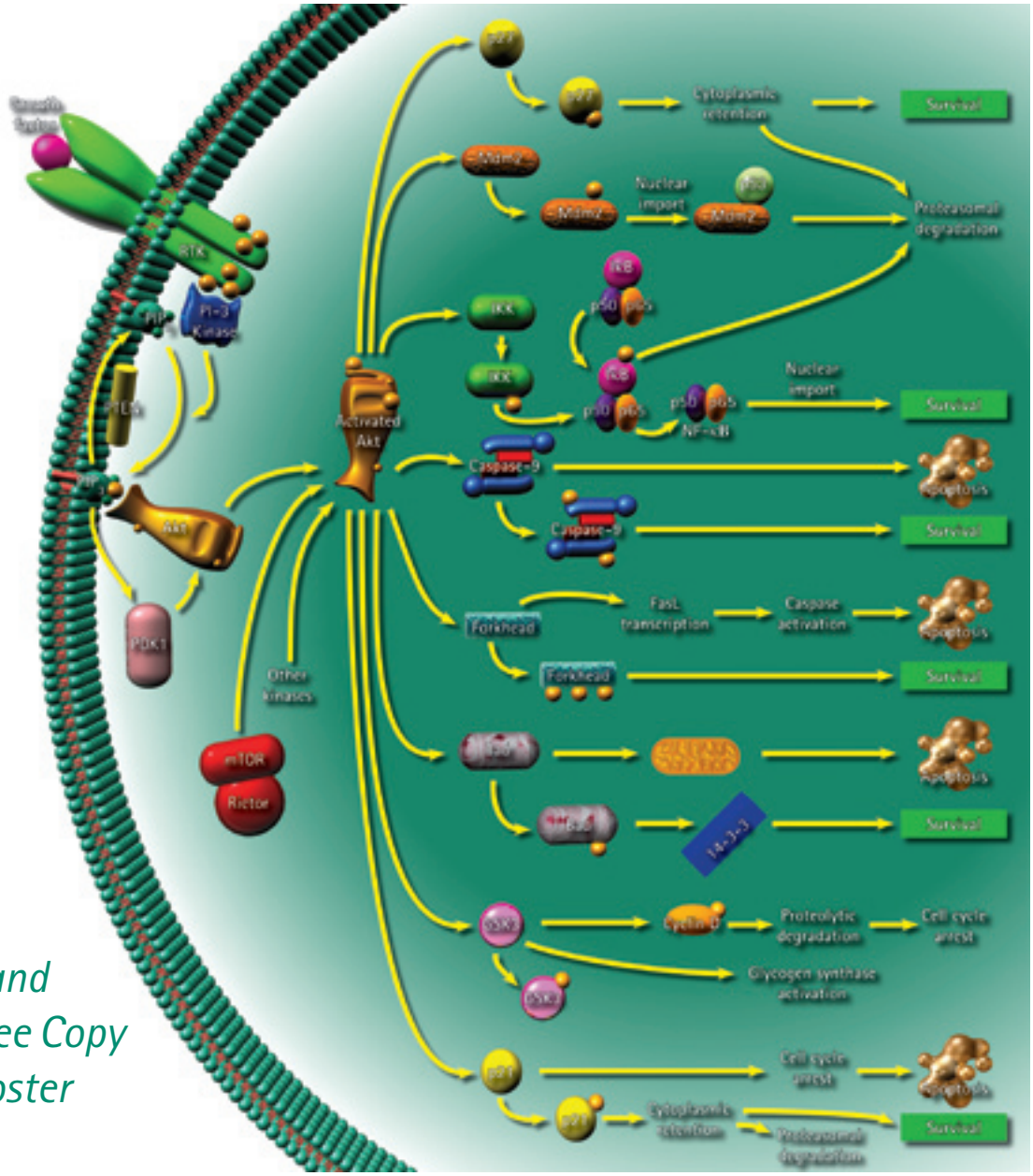
Akt (protein kinase B), a serine/threonine kinase, has emerged as a critical enzyme in signal transduction pathways involved in cell proliferation, apoptosis, angiogenesis, and diabetes. In mammals three isoforms of Akt (α, β, γ or Akt 1, 2, 3) are reported that exhibit a high degree of homology, but 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. The PH domain in the N-terminal region of Akt interacts with 3´-phosphoinositides and helps to recruit Akt to the plasma membrane.

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 primarily by mTOR/richtor complex (mTORC2). DNA-PK and $PKC_{βII}$ 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 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 down-regulation of Akt, which predisposes cells to rapid apoptosis in response to stress signals. Under certain circumstances Akt



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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	Price
Anti-Akt1 (Ab-1) (135-145) Rabbit pAb	PC510	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 134-145 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 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 Akt1. 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. 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. **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	

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 and Kits

Product	Cat. No.	Comments	Size	Price
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	
Anti-PTEN Mouse mAb (EMD-15E10)*	AP1041	Monoclonal IgG_1, purified. Immunogen used was a full length recombinant human PTEN expressed in Sf9 cells. Recognizes the ~55 kDa PTEN protein in MCF-7 cells. **ELISA, IP,**	50 µg	
Anti-PTEN Mouse mAb (EMD-4B8)*	AP1042	Monoclonal IgG_1, purified. Immunogen used was a full length recombinant human PTEN expressed in Sf9 cells. Recognizes the ~55 kDa PTEN protein in MCF-7 cells. **IB**	50 µg	
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	
PRAS40 ELISA Kit	CBA066	Detects and quantifies the level of PRAS40 (Proline-Rich AKT Substrate of 40 kDa) independent of its phosphorylation state. PRAS40 is a 14-3-3 binding protein that is a direct substrate of Akt. PRAS40 is believed to influence protein interactions, nuclear transport, and enzyme activities.	1 kit	
PhosphoDetect™ PRAS40 (pThr^{246}) ELISA Kit	CBA067	Detects and quantifies the level of PRAS40 (Proline-Rich AKT Substrate of 40 kDa) protein that is phosphorylated at Thr^{246} in mouse, human, and rat samples. PRAS40 is a 14-3-3 binding protein that is a direct substrate of Akt. PRAS40 is believed to influence protein interactions, nuclear transport, and enzyme activities.	1 kit	

ELISA: enzyme-linked immunosorbent assay; **IB**: immunoblotting; **IC**: immunocytochemistry; **IF**: immunofluorescence; **IP**: immunoprecipitation; **PS**: paraffin sections; **10T**: 10 tests by Western miniblots

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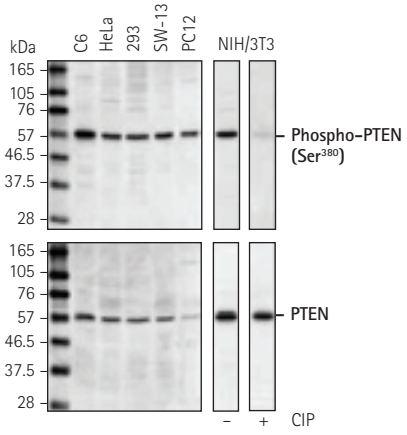
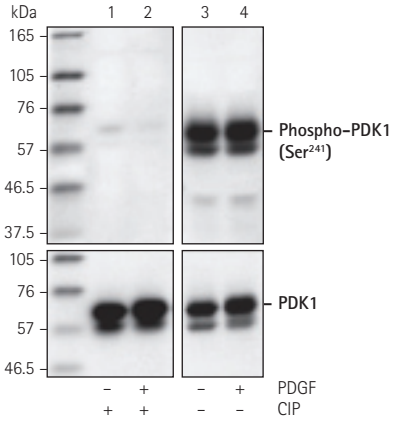


Detection of human PTEN phosphorylated on Ser^{380} by immunoblotting. Lysates from C6, HeLa, 293, SW-13, PC-12 (all untreated) and NIH-3T3 cells untreated or treated with calf intestinal alkaline phosphatase (CIP). Primary antibody Phospho-Detect™ Anti-PTEN (pSer^{380}), Rabbit pAb (Cat. No. ST1072, 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: PhosphoDetect™ Anti-PDK1, (pSer^{241}), Rabbit pAb (Cat. No. ST1073) or Anti-PDK1 (bottom panel).

Recombinant Akt and Akt Substrates

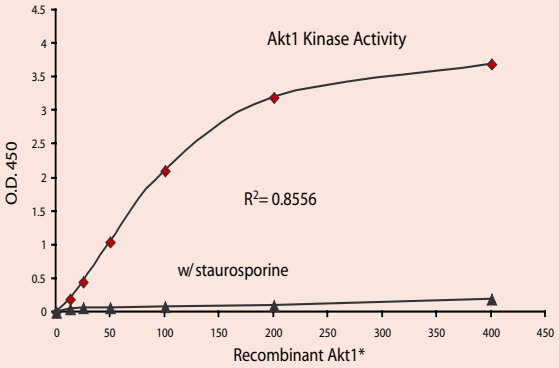
Product	Cat. No.	Comments	Size	Price
Akt1, His•Tag®, Activated, Human, Recombinant, *S. frugiperda*	124006	A purified recombinant human Akt1 expressed in *Spodoptera frugiperda* cells. Highly active form of Akt1 suitable for labeling Akt substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Specific activity: ≥20,000 units/mg protein. *Purity:≥95% by SDS-PAGE.* M.W. 60,000.	20 µg	
Akt2, GST-Fusion Protein, Active, Human, Recombinant	124021	Human, recombinant Akt2 consisting of amino acids 1-119 (minus the PH domain) expressed as a GST fusion protein (N-terminal) using a baculovirus expression system. This recombinant protein contains the S473D and T308E mutations.	20 µg	
Akt2, GST-Fusion Protein, Active, Human, Recombinant, *S. frugiperda*	124022	Full-length, human, recombinant Akt3 fused to GST at the N-terminus and expressed Sf9 cells using a baculovirus expression system.	5 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	

Akt Assay Kits and Related Kits

Product	Cat.No.	Comments	Size	Price
Akt Activity Immunoassay Kit	124007	A non-radioactive assay kit for measuring Akt activity in cell lysates or tissue extracts from human, mouse, and rat. 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	
PhosphoDetect Akt (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 be suitable for use with mouse and rat.	1 kit	
PhosphoDetect Akt (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, it is also suitable for use with mouse and rat cells.	1 kit	

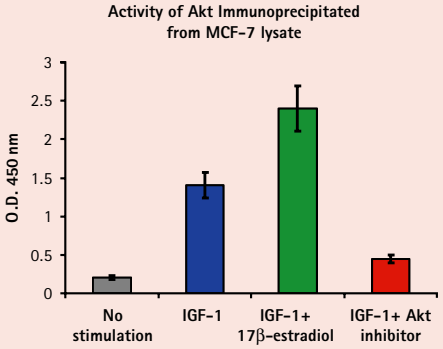
K-LISA™ Akt Activity Kit

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, Akt3, SGK, and MSK1.



(*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 action.

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, 124019, 124020, 252740, 476880)

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, 124019, 124020)

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 Inhibitors

Product	Cat. No.	Comments	Size	Price
Akt Inhibitor	124005	A PI analog, prevents PIP_3 formation and binding to Akt. Inhibits Akt (IC_{50} = 5.0 µM) and PI 3-K (IC_{50} = 83 µM)	1 mg	
Akt Inhibitor II (SH-5)	124008	A 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	A 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	A cell-permeable, ATP-competitive inhibitor of a kinase upstream of Akt, but downstream of PI 3-K.	1 mg 5 mg	
InSolution™Akt Inhibitor IV	124015	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	A cell-permeable inhibitor of Akt. Blocks 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	A15-mer peptide that directly binds Akt PH domain (k_d=18 µM), preventing PI binding.	2 mg	
Akt Inhibitor VII, *TAT-Akt-in*	124014	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	A cell-permeable inhibitor of Akt. 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 vitro* kinase assays).	1 mg	
InSolution™ Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	124017	A 10 mM (1 mg/181 µl) solution of Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2 (Cat. No. 124018) in DMSO.	1 mg	
NEW Akt Inhibitor IX, API-59CJ-OMe	124019	A cell-permeable ellipticine compound that potently and selectively inhibits cell growth and induces apoptosis in human endometrial cancer cells with elevated Akt levels. Exhibits minimal effect on cells with low Akt activity.	5 mg	
NEW Akt Inhibitor X	124020	A cell-permeable, selective inhibitor of the phosphorylation of Akt and its *in vitro* kinase activity (complete inhibition < 5 µM) with minimal effect on PI 3-K, PDK1, or SGK1. Unlike Akti1/2 (Cat. No. 124018), the mode of inhibition is not PH domain-dependent.	5 mg	
NEW Akt Inhibitor XI	124028	A cell-permeable copper complex (Cu^{2+}/Cu^{+} redox couple in the range of +0.28 to +0.35 V) that interacts with both the PH and the kinase domains of Akt and potently inhibits its kinase activity (IC_{50} = 100 nM). Inhibits tumor growth both in cultured cells *in vitro* (IC_{50} ranges from 10 to 34 µM) and in mice *in vitro* (25 mg/kg, iv) without any apparent adverse effect to the animals.	5 mg	
NEW (-)-Deguelin, *Mundulea serica*	252740	A cell-permeable, potent inhibitor of mitochondrial bioenergetics (IC_{50} = 6.9 nM for NADH: ubiquinone oxidoreductase activity in bovine heart ETP). Promotes mitochondrial permeability transition. Selectively blocks Akt activation with minimal effects on MAPK signaling. Also shown to activate AMPK activity and inhibit COX-2 expression.	5 mg	
NEW Naltrindole, Hydrochloride	476880	A cell-permeable inhibitor of Akt signaling. Decreases phosphorylation level of PDK1, Akt, FKHR/AFX, GSK-3β, and inhibits Akt-dependent cell growth in small cell lung cancer (SCLC) cell line (IC_{50} = 25, 40, and 55 µM in NCI-H69, NCI-H345, and NCI-H510, respectively).	5 mg	
NEW PDK1/Akt/Flt Dual Pathway Inhibitor	521275	A cell-permeable inhibitor of PDK1 and Akt in *in vitro* kinase assays. Blocks phosphorylation of Akt at both Ser^{473} and Thr^{308}.	5 mg	

mTOR

mTOR (**m**ammalian **T**arget **o**f **R**apamycin) was originally identified in *Saccharomyces cerevisiae,* where mutations of the protein kinase TOR confer rapamycin resistance. mTOR is conserved evolutionarily and it integrates nutrient and growth factor-derived signals to control cell growth. mTOR is a large (>250 kDa) class IV PI-3 kinase family member with protein kinase activity, but lacks any lipid kinase activity. mTOR forms a complex with the 12 kDa cytosolic protein, FKBP-12 and rapamycin that functions to arrest the cell cycle in the G1 phase. mTOR exists as two complexes, mTORC1, and mTORC2. mTORC1, composed of mTOR, GβL, and Raptor regulate cell growth and protein translation. mTORC2, composed of mTOR, GβL, Rictor and mSin, regulates actin polymerization and phosphorylates Akt on Ser^{473}. Biomarkers indicate that the mTOR pathway is hyperactive in certain types of cancers, suggesting that mTOR could be an attractive target for cancer therapy. Also, there is sufficient evidence to link deregulated protein synthesis to tumorigenesis via the translation initiation factor complex eIF-4F. Activated mTOR may provide tumor cells with a growth advantage by promoting protein synthesis, which is the best-described physiological function of mTOR signaling. mTOR regulates Akt activity, a crucial downstream effector in the PI-3K–PTEN pathway, which controls cell proliferation and survival. Targeting this function of mTOR may also have therapeutic potential.

mTOR Related Antibodies

Product	Cat. No.	Comments	Size	Price
PhosphoDetect™ Anti-p70S6K(pThr^{389}) Rabbit pAb	PK1015	Polyclonal IgG, immunoaffinity purified. Immungen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Thr^{389} phosphorylation site of human p70S6K. Recognizes the ~60 kDa p70S6 kinase protein phosphorylated on Tyr^{389} in MCF7 cells. **ELISA, IB**	50 µg	
Anti-4EBP1/PHAS-I (101-118) Rabbit pAb	516676	Polyclonal IgG, immunoaffinity purified. Immungen used was a synthetic peptide (SPEDKRAGGEESQFEMDI) corresponding to amino acids 101-118 of human PHAS-I, conjugated to KLH. Recognizes the ~19-25 kDa multiple phosphorylation states of native and recombinant PHAS-I protein in human, mouse, and rat. **GS, IB**	100 µg	
Anti-AMPKα-2 Rabbit pAb	ST1089	Polyclonal IgG, immunoaffinity purified. Immungen used was a synthetic peptide corresponding to a portion of mammalian AMP-activated protein kinase, a-2 (AMPKa-2) subunit encoded within exon 7. Recognizes the ~64 kDa AMPK α-2 protein in aortic endothelial cells in bovine, human, and rat. **IB, IP**	50 µg	
PhosphoDetect™ Anti-eIF-4E (Ab-1) (pSer^{209}) Rabbit pAb	PC639	Polyclonal IgG, immunoaffinity purified. Immungen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{209} phosphorylation site of human eIF-4E. Recognizes the ~25 kDa eIF-4E protein phosphorylated at Ser^{209} in human, mouse, rabbit, and rat. **IB**	10 T	
Anti-LKB1 (120-160) Rabbit pAb	ST1092	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic peptide located between amino acid residues 120-160 of human LKB1. Detects the ~47 kDa LKB1 protein, an evolutionarily conserved serine/threonine kinase that may function as a tumor suppressor in human. **IB**	50 µg	
Anti-p70S6 Kinase Rabbit pAb	ST1046	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic peptide corresponding to the C-terminus of p70S6 kinase. Recognizes the ~70 kDa p70S6 kinase protein in serum-starved rat L6 myoblasts in human, mouse, and rat. **IB**	100 µg	
Anti-Raptor Rabbit pAb	ST1048	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic peptide corresponding to amino acids encoded within exon 26 of human raptor. Recognizes the ~150 kDa raptor protein in human, mouse, and rat. **IB**	100 µg	
PhosphoDetect™ Anti-mTOR (pSer^{2448}) Rabbit pAb	PS1020	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acids surrounding Ser^{2448} in human TOR. Detects ~290 kDa mTOR protein phosphorylated on Ser^{2448} in EGF-treated HEK293 cells in human. **IB**	50 µg	
Anti-mTOR/FRAP (Ab-2) Mouse mAb (22C2)	OP97	Monoclonal, purified. Immunogen used was a synthetic peptide corresponding to amino acids 230-240 of human TOR. Recognizes the ~290 kDa mTOR protein in HEK293 cells in human. **IB, IP**	100 µg	
Anti-TSC1 (Tuberous Sclerosis 1) Rabbit pAb	AP1032	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic peptide corresponding to amino acids 1100-1164 of human TSC1. Detects the ~120 kDa tuberous sclerosis 1 protein (TSC1) protein in HeLa, MEF, and U2OS cells in human, and mouse. **IB, IP**	50 µg	
PhosphoDetect™ Anti-Tuberin/TSC2 (pThr^{1462}) Rabbit pAb	ST1084	Polyclonal IgG, protein A and immunoaffinity purified. Immunogen used was a synthetic peptide corresponding to amino acids surrounding the Thr^{1462} phosphorylation site of human Tuberin/TSC2. Recognizes the ~200 kDa tuberin/TSC2 protein phosphorylated at Thr^{1462} in human, and mouse. **IB**	50 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

mTOR Inhibitors

Product	Cat. No.	Comments	Size	Price
Rapamycin	553210	Anti-fungal and immunosuppressant. Inhibits mTOR by binding to FK506-binding protein-12. Selectively inhibits the phosphorylation and activation of p70 S6 kinase (IC_{50} = 50 pM). Prevents the translational activation of IGF-II. Shown to inhibit later signaling events such as $p110^{Rb}$ phosphorylation, $p34^{cdk1}$ kinase activation, and cyclin A synthesis. Reported to induce apoptosis in a murine B cell line, to inhibit lympho-kine-induced cell proliferation at the G_1 phase, and to irreversibly arrest *Saccharomyces cerevisiae* G_1 phase.	100 µg 1 mg	
InSolution™ Rapamycin	553211	Supplied as a 5 mM (500 µg/109 µl) solution of Rapamycin (Cat. No. 553210) in DMSO.	500 µg	
NEW PI-103	528100	A cell-permeable pyridinylfuranopyrimidine compound that acts as a potent and ATP-competitive inhibitor of DNA-PK, PI 3-K, and mTOR (IC_{50} = 2, 8, 88, 48, 150, 26, 20, and 83 nM for DNA-PK, p110α, p110β, p110δ, p110γ, PI 3-KC2β, mTORC1, and mTORC2, respectively). It inhibits ATR and ATM only at much higher concentrations (IC_{50} = 850 and 920 nM, respectively) and exhibits little activity towards a panel of more than 40 other kinases even at concentrations as high as 10 µM. Shown to effectively block PI 3-K/Akt signaling and cell proliferation in glioma cell lines both *in vitro* and *in vivo*.	1 mg 5 mg	

Related Proteins

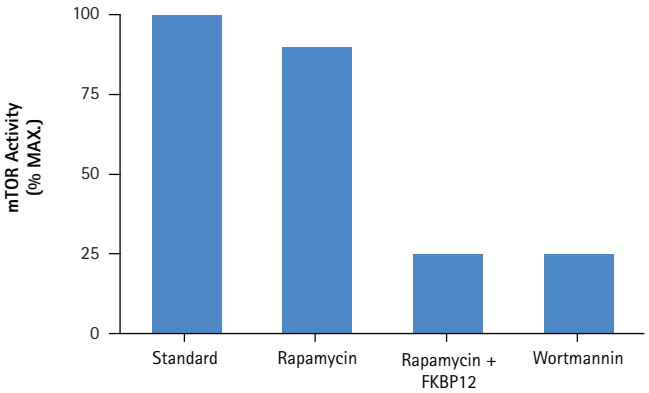
Product	Cat. No.	Comments	Size	Price
4EBP1/PHAS-I, Rat, Recombinant	516675	A 117-amino acid protein substrate that has been shown to be an excellent substrate for MAP kinase, p38 kinase, PKC, and JNK. Phosphorylation of PHAS-I increases in response to insulin but not to cAMP stimulation of adipocytes. An excellent substrate for MAP kinase both *in vivo* and *in vitro*.	250 µg	
p70S6K, Human, Recombinant	506182	A full-length, active, recombinant, human p70 S6 kinase.	5 µg	

K-LISA™ mTOR Activity Kit

A 96-well ELISA-based activity assay for measuring the kinase activity of purified mTOR or mTOR immunoprecipitated from cell lysates. The kit utilizes a p70S6K-GST fusion protein as a specific mTOR substrate. The mTOR substrate is first bound to the wells of a glutathione-coated plate, followed by the addition of the mTOR containing samples in the presence of ATP. mTOR phosphorylates p70S6K at Thr^{309}. The phosphorylated substrate is detected with anti-p70S6K-T^{389} antibody followed by detection with an HRP-antibody conjugate and TMB substrate. Useful for *in vitro* mTOR inhibitor screening and for assessing the regulation of mTOR cell signaling.

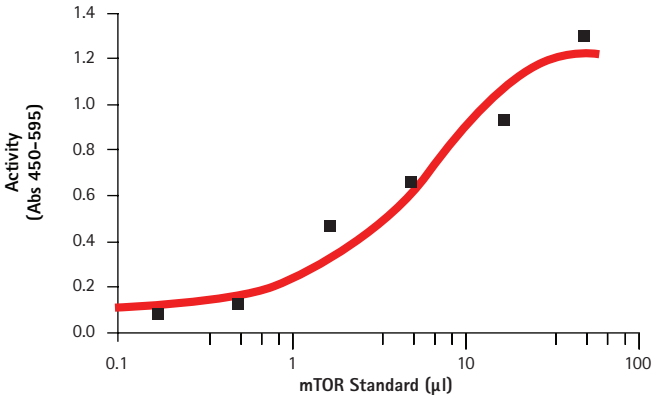
Sold under exclusive license of allowed U.S. Patent Application 20040191836.

Cat. No. CBA055 1 kit



Rapamycin + FKBP12 Inhibition of mTOR Kinase Activity

The activity of the mTOR Standard, (50 µl) was determined in the presence of either Rapamycin (20 µM; Cat. No. 553210 or 553211), Rapamycin (20 µM) + GST-FKBP12 (37 µg/ml), or Wortmannin (10 µM; Cat. No. 681675; also included with the kit).



Activity of mTOR Standard

The mTOR Standard supplied in the kit is an enriched rat brain fraction isolated using proprietary methods. The mTOR standard phosphorylates p70S6K specifically on Thr^{389}, and is inhibited by FKBP12/Rapamycin [(20 µM; Cat. No. 553210 or 553211)], a specific inhibitor of the mTOR/Raptor complex, as well as wortmannin (10 µM; Cat. No. 681675; also included with the kit), a more general PI-3K inhibitor. Addition of Wortmannin serves as a positive control for inhibitor analysis. Inhibition profiles can be generated based on mTOR activity in the presence and absence of test inhibitor(s).

Phosphoinositide 3-Kinases (PI 3-K)

The PI 3-kinases are ubiquitous, heterodimeric enzymes that play a pivotal role in the regulation of many cellular processes, including cell growth, motility, proliferation, and survival. They are dual-specificity enzymes capable of phosphorylating phosphoinositides. PI 3-kinases are divided into three classes. Class I kinases were the first to be characterized and include receptor-regulated heterodimeric enzymes consisting of a 110 kDa catalytic subunit and an 85 kDa regulatory subunit (p85/ p110α; p85/p110β; p101/P110γ). p85 subunit binds and integrates signals from various cellular proteins, including membrane tyrosine kinase-linked receptors. PI 3-Kinases can use PI, PI (4)P and PI (4,5)P_2 as substrates *in vitro*. The major substrate *in vivo* appears to be PI (4,5)P_2. The members of this class are sensitive to wortmannin. Class II PI-3 kinases are monomeric and lack adapter subunits. They can phosphorylate PI and PI(4)P *in vitro* and show variable responses to wortmannin. This class of enzymes contains a C-2 domain at the C-terminal region that binds phospholipids in a Ca^{2+}-dependent manner. They participate in integrin signaling in platelets. Class III PI 3-kinases are heterodimeric enzymes consisting of adaptor (p150) and catalytic (Vps34) subunits. Vps34 can phosphorylate PI(3)P and are believed to play a role in vesicle trafficking and autophagy. The human homologue of Vps34 is reported to be sensitive to wortmannin and participates in the regulation of endocytic membrane trafficking.

Activated PI 3-kinase phosphorylates phosphoinositol (PI) substrates to produce PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3. These molecules act as second messengers and recruit the PI 3-K-dependent serine/threonine kinases (PDK1) and Akt from the cytoplasm to the plasma membrane. Lipid binding and membrane translocation lead to conformational changes in Akt, which gets phosphorylated on Thr^{308} in the activation loop by PDK1, and Ser^{473} in the hydrophobic phosphorylation motif by mTORC2. This dual phosphorylation causes full activation of the enzyme. Inhibitors of PI 3-kinase and over-expression of dominant negative PI 3-kinase mutants are shown to block many of the physiological responses of a cell to insulin, indicating that PI 3-kinase lies upstream of these events.

Dysregulated PI 3-K signaling pathway has been reported in a variety of human tumors. Over 30% of various solid tumors are reported to contain mutations in the catalytic unit of their PI 3-K. Functional analyses of the catalytic subunit of PI 3-K mutations indicate that these mutations abnormally increase its enzymatic activity, stimulate AKT signaling, allow growth factor-independent growth as well as increasing cell invasion and metastasis. Hence, PI 3-kinase is becoming an attractive target for drug development, not only in the areas of cancer and other proliferative diseases, but also in the treatment of inflammatory and immunological conditions.

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PI 3-Kinase, Antibodies

Product	Cat. No.	Comments	Size	Price
Anti-PI-3-Kinase Mouse mAb (AB6)	528107	Monoclonal, purified. Purified by ammonium sulfate precipitation and protein A. Immunogen was a recombinant human p85α expressed in *E. coli*. Recognizes the ~ 85 kDa p85α regulatory subunit of PI 3-kinase in human, mouse, and rat. Does not cross-react with p85β. **IB, IC, IP**	100 µg	
Anti-PI-3-Kinase p110δ, C-Terminal (1026-1044) Rabbit pAb	526553	Polyclonal IgG, immunoaffinity purified. Immunogen was a synthetic peptide [(C)SWKTKVNWLAHNVSKDNRQ) corresponding to a distinct C-terminal region of human phosphatidylinositol 3-kinase p110δ, conjugated to KLH. Recognizes a ~110 kDa PI-3 kinase p110δ protein in human. **IB, IC**	100 µl	
PI3K NT-frag., His•Tag® fusion	526555	A recombinant, human protein containing the N-terminal 483 aa of human PI 3-K with one mutation (N483K) expressed in *E. coli* with N-terminal His•Tag® and S•Tag™ sequences. Suitable for use as a substrate for protein tyrosine kinases in *in vitro* assays, but has no intrinsic kinase activity. (It is not sensitive to nanomolar levels of wortmannin.)	50 µg	

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

PI 3-Kinase Inhibitors

Product	Cat. No.	Comments	Size	Price
ET-18-OCH_3	341207	A selective cell-permeable inhibitor of phosphatidylinositol-specific PLC (IC_{50} = 15 µM) but does not inhibit phosphatidylcholine-specific PLC or PLD.	5 mg	
DNA-PK Inhibitor III	260962	A cell-permeable, potent, selective, ATP-competitive inhibitor of DNA-PK (IC_{50} = 120 nM) and PI 3-Kinase catalytic subunit p110β (IC_{50} = 135 nM). Inhibits PI 3K p110α, p110γ, and p110δ only at much higher concentrations (IC_{50} = 1.4, 0.88, and 1.0 µM, respectively).	1 mg	
LY 294002	440202	A cell-permeable, 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	Supplied as a 10 mM (1 mg/325 µl) solution of LY 294002 (Cat. No. 440202) in DMSO.	1 mg	
LY 303511	440203	A cell-permeable negative control for the PI 3-kinase inhibitor, LY 294002 (Cat. No. 440202). Contains a single atom substitution in the morpholine ring compared to LY 294002. Does not affect PI 3-kinase activity even at concentrations ≥100 µM.	1 mg	
NEW PI-103	528100	A cell-permeable pyridinylfuranopyrimidine compound that displays antitumor properties in a mouse model of human glioma. Acts as a potent and ATP-competitive inhibitor of DNA-PK, PI 3-K and mTOR (IC_{50} in nM = 2, 8, 88, 48, 150, 26, 20 and 83 for DNA-PK, p110α, p110β, p110δ, p110γ, PI 3-KC2β, mTORC1 and mTORC2, respectively) and exhibits little activity towards a panel of more than 40 other kinases, even at concentations as high as 10 µM. Reported to block PIP_3 production, insulin signaling and Akt-phosphorylation, and to inhibit cell proliferation and induce cell-death.	1 mg 5 mg	
NEW PI 3-Kγ Inhibitor	528106	A cell-permeable thiazolidinedione compound that acts as a potent, selective, and ATP-competitive inhibitor of PI 3-Kγ (K_i = 7.8 nM; IC_{50} = 8 nM, 60 nM, 270 nM, 300 nM for p110-γ, α, β and δ-isoforms, respectively). Does not affect the activity of a wide panel of kinases, receptors, enzymes, and ion channels even at concentrations as high as 1 µM.	5 mg	
NEW PI 3-Kγ Inhibitor II	528108	A cell-permeable, potent and ATP-competitive inhibitor of PI 3-Kγ (K_i = 180 nM; IC_{50} = 250 nM). Exhibits great selectivity over PI 3-Kα (IC_{50} = 4.5 µM), PI 3-Kβ and δ (IC_{50} >20 µM).	5 mg	
NEW PI 3-Kγ/CKII Inhibitor	528112	A cell-permeable, potent and ATP-competitive dual specific inhibitor of PI 3-Kγ;/CKII (IC_{50} = 20 nM).	5 mg	
Quercetin, Dihydrate	551600	An inhibitor of PI 3-kinase (IC_{50} = 3.8 µM) and phospholipase A_2 (IC_{50} = 2 µM).	100 mg	
Wortmannin	681675	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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