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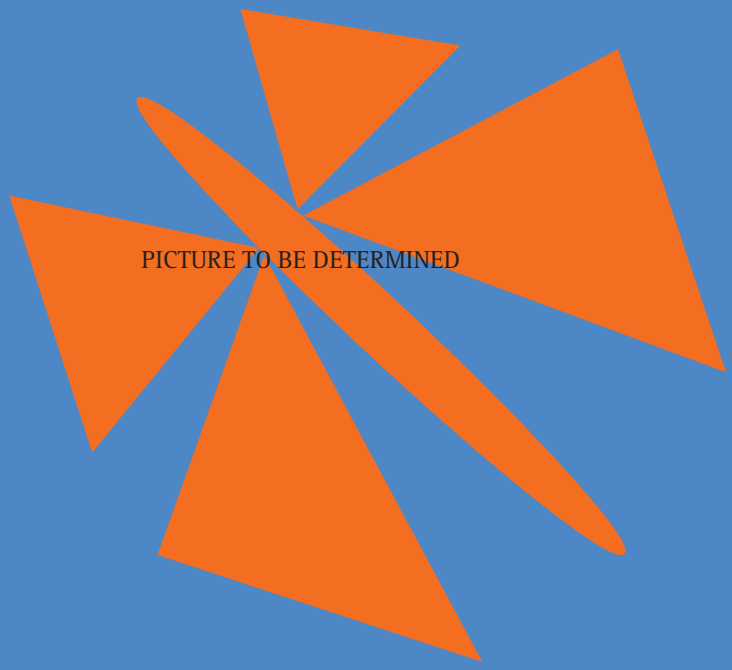
Calbiochem®

Biologics

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



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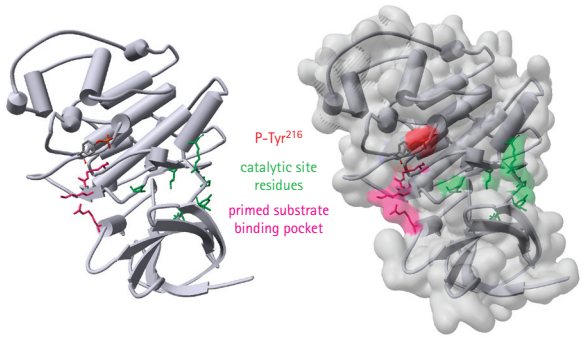
Inside this Issue...

EMD

Glycogen Synthase Kinase-3: Its Signaling Role in Development and Disease

By Chandra Mohan, EMD Biosciences

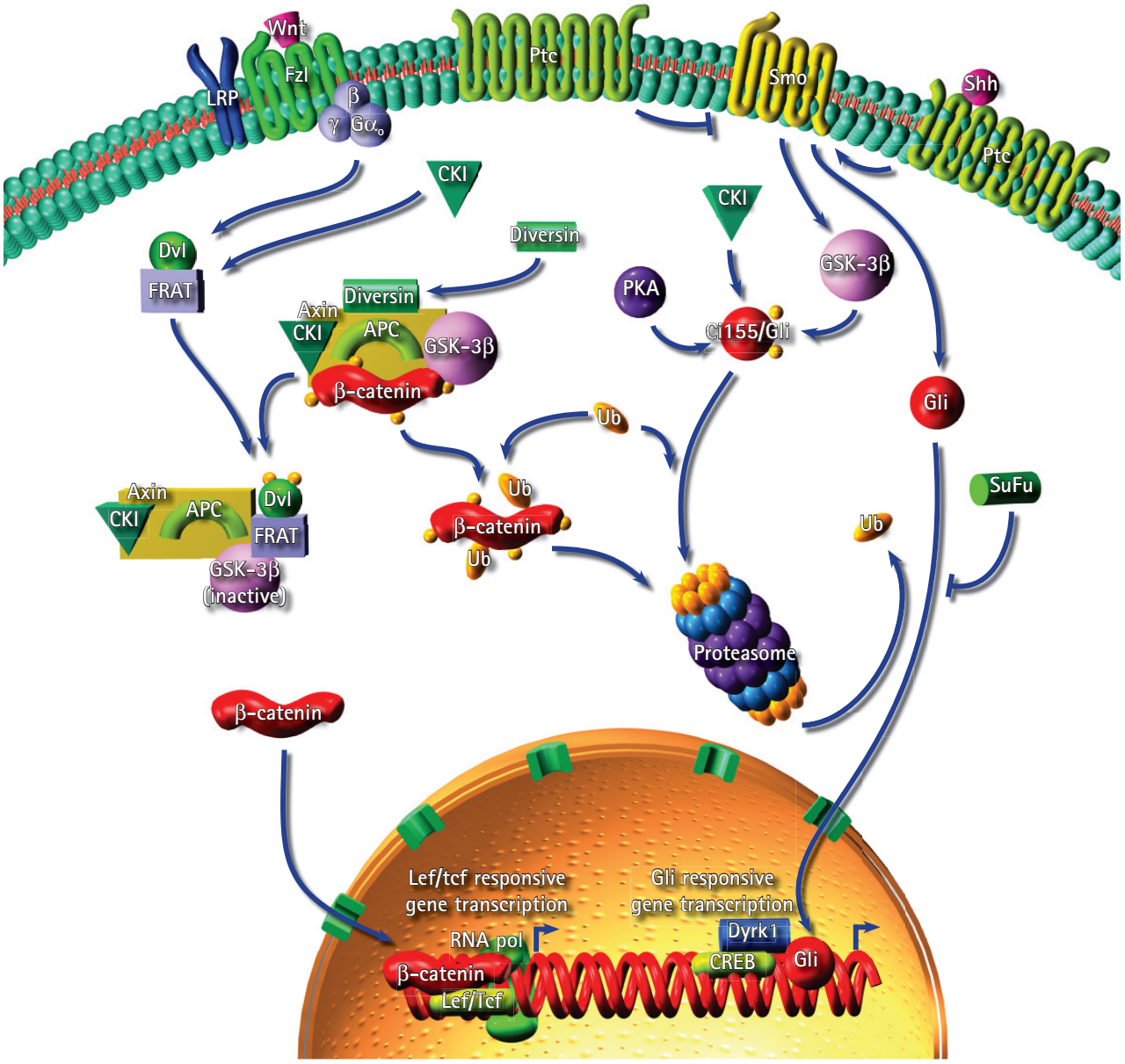
Glycogen synthase kinase-3 (GSK-3), a multifunctional serine/threonine kinase, is a key regulator of numerous signaling pathways. Two isoforms of GSK-3 are reported in mammals: a 51 kDa GSK-3α and a 47 kDa GSK-3β. The GSK-3α contains a glycine-rich extension at its N-terminal. These two isoforms exhibit about 98% homology in their kinase domains, but share only about 36% identity in the last 76 C-terminal amino acid residues. A minor (~15% of total) splice variant of GSK-3β, GSK-3β2, has also been identified, which contains a 13-residue insert within the kinase domain. It exhibits reduced kinase activity towards the tau protein compared with 'unspliced' GSK-3β. GSK-3β2 is localized primarily to neuronal cell bodies, unlike unspliced GSK-3β that is also found in neuronal processes.



GSK-3 is normally active in cells and is regulated through inhibition of its activity. GSK-3 shows a preference for target proteins that are pre-phosphorylated at a `priming' residue located C-terminal to the site of GSK-3 phosphorylation. Priming phosphorylation, although not absolutely required, enhances the efficiency of phosphorylation of most GSK-3 substrates. Phosphorylation of a Thr residue in the activation loop (T-loop) is considered to be essential for several protein kinases, such as Cdk2, p38γ, and ERK2 that are closely related to GSK-3. This phosphorylation at Thr is also required by p38γ and ERK2 to open up the catalytic site for substrate access. The T-loop of GSK-3α is phosphorylated at Tyr^{279} and GSK-3β at Tyr^{216}, which play a role in forcing open the substrate-binding site of the enzyme. Uniquely the T-loop of GSK-3 does not undergo any Thr phosphorylation. The function of missing pThr in the T-loop of GSK-3 is carried out by the phosphorylated residue of a primed substrate that binds to a positively charged pocket consisting of Arg^{96}, Arg^{180}, and Lys^{205} (for GSK-3β). This arrangement optimizes the orientation of the kinase domain and places the substrate at the proper position within the catalytic groove for phosphorylation to take place.

GSK-3β is constitutively active in resting cells and treatment of cells with an agent, such as insulin, is shown to cause GSK-3 inactivation through a PI 3-kinase (PI 3-K)-dependent mechanism. PI 3-K-induced activation of PKB/Akt results in phosphorylation of Ser^{21} on GSK-3α and Ser^{9} on GSK-3β, which inhibit GSK-3 activity. The phosphorylated N-terminus becomes a primed pseudosubstrate that occupies the positive binding pocket and the active site of the enzyme and acts as a competitive inhibitor for true substrates. This prevents phosphorylation of substrates. Arg^{96} is shown to be a crucial component of the positive pocket that binds primed substrates. Arg^{96} to Ala^{96} mutation disrupts the pocket in a way that primed substrates can no longer bind and, hence, the enzyme remains active. Also, Ser^{9}-phosphorylated pseudosubstrate is no longer capable of inactivating the enzyme. Small molecule inhibitors that fit in the positively charged pocket of the kinase domain of GSK-3β are useful for selectively inhibiting primed substrates. Several known GSK-3 substrates participate in a wide spectrum of cellular processes, including glycogen metabolism, transcription, translation, cytoskeletal regulation, intracellular vesicular transport, cell cycle progression, and apoptosis. Phosphorylation of these substrates by GSK-3β usually has an inhibitory effect.

GSK-3β plays a key inhibitory role in the Wnt signaling pathway. Wnt genes encode a large family of secreted, cysteine-rich proteins that are important in development and in maintenance of adult tissues. Abnormalities in Wnt signaling are reported to promote both human degenerative diseases and cancer. Several groups have shown that β-catenin is a primed substrate for GSK-3β, with casein kinase I (CKI) acting as the priming kinase. In this capacity CKI functions as a negative regulator of Wnt signaling since it promotes GSK-3 function. In unstimulated cells, CKI phosphorylates β-catenin on Ser^{45}, priming it for further phosphorylation on $Ser^{41, 35, and 33}$ by GSK-3β in a sequential manner, thereby allowing β-catenin to be ubiquitinated for proteasomal degradation. It has been



suggested that ankyrin repeat protein, Diversin, may help recruit CKI to the destruction complex. Wnt stimulation activates the receptor Frizzled, which then signals through Dishevelled (Dvl) to inactivate β-catenin phosphorylation. Unphosphorylated β-catenin translocates to the nucleus where it transactivates genes regulated by TCF/LEF transcription factors. Another key player in the regulation of the Wnt signaling pathway is GBP/FRAT, a GSK-3-binding protein. Binding of GBP/FRAT to GSK-3β prevents GSK-3β from binding to axin and thus it interferes with β-catenin phosphorylation. GBP/FRAT also plays a significant role in the nuclear export of GSK-3β. This suggests that GBP/FRAT may be involved in regulating the access of GSK-3 to substrates partitioned between the nucleus and the cytoplasm. Any mutation that prevents the binding of GSK-3β to GBP/FRAT causes nuclear localization of GSK-3β. A small peptide derived from FRAT, FRATtide, is reported to prevent axin-GSK-3 interaction and prevents phosphorylation of both axin and β-catenin. In the Wnt signaling pathway GSK-3β appears to be insulated from regulators of GSK-3β that lie outside of the Wnt pathway. Insulin signaling that leads to inhibition of GSK-3 via phosphorylation at either Ser^{9} or Ser^{21} does not cause accumulation of β-catenin. Mutations in β-catenin that prevent its phosphorylation by GSK-3β are common in skin, colon, prostate, liver, endometrial, and ovarian cancers.

GSK-3β also plays a significant role in Hedgehog signaling. Sonic Hedgehog (Shh) has been implicated in several embryonic developmental processes and it displays inductive, proliferative, neurotrophic, and neuroprotective properties. Response to Shh signaling is controlled by two transmembrane proteins, the patched (Ptc), a twelve-span transmembrane protein, and Smoothened (Smo), a seven transmembrane receptor protein. Ptc acts an inhibitor of Smo activation. Binding of Shh to Ptc lifts the inhibitory effect on Smo, leading to the activation of the Shh signal-

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ing cascade. Wnt and Shh often work in concert to set the embryonic development pattern. The Wnt pathway uses β-catenin to transduce its signals to the nucleus, whereas the Shh pathway employs a 155 amino acid protein, Cubitus interruptus (Ci155) in *Drosophila* or Gli in mammals. In vertebrates three Gli proteins (Gli1, Gli2, and Gli3) have been reported, Gli1 and Gli2 function primarily as activators and Gli3 has a repressor role. In the absence of any Shh signal, Ci is targeted for proteolysis, which generates a truncated 75-residue amino acid form (Ci75), which functions as a transcriptional repressor. GSK-3β, in combination with CKI and the priming PKA, phosphorylates Ci155 (and probably Gli) and targets it for proteolytic processing in the absence of a Shh signal. Activation of Shh signaling results in translocation of Ci155/Gli to the nucleus, where it activates Hh target genes. Although GSK-3β phosphorylation of the mammalian homologs of Ci has yet to be reported, they all contain multiple GSK-3 consensus sites next to PKA sites. Suppressor of Fused (SuFu) interacts directly with Gli proteins, repressing Shh signaling while Dyrk1 acts by a distinct pathway to stimulate Gli1 activation of transcription. Over signaling by Shh appears to be involved in the initiation and propagation of some tumors of the muscle, skin and nervous system.

Abnormalities in pathways that use GSK-3 as a regulator have been linked to several disease conditions. Hence, GSK-3 has emerged as a potential therapeutic target, particularly in non-insulin-dependent diabetes mellitus, Alzheimer's disease, developmental disorders, and cancer. Several new GSK-3 inhibitors have recently been developed, most of which act in an ATP competitive manner. Inhibitors belonging to aloisines, the paullones, and the maleimide families, have shown promise as therapeutic agents. Due to its involvement in multiple pathways, selectivity of GSK-3 inhibition is an important factor in the development of inhibitors for therapeutic applications.

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NEW GSK-3 Inhibitors

Product	Cat. No.	Comments	Size	Price
1-Azakenpaullone	191500	A potent, ATP-competitive inhibitor of GSK-3β (IC_{50} = 18 nM).	1 mg	95
Alsterpaullone, 2-Cyanoethyl	126871	An Alsterpaullone (Cat. No. 126870) derivative that acts as a highly potent, ATP-competitive, selective inhibitor of GSK-3β and Cdk1/cyclin B (IC_{50} = 800 pM and 230 pM, respectively).	1 mg	135
GSK-3β Inhibitor IX	361553	A cell-permeable, potent, specific, and ATP-competitive inhibitor of GSK-3β (K_i = 25 nM). Has only a trivial effect on a panel of 79 commonly studied protein kinases	1 mg	128
GSK-3 Inhibitor IX	361550	A cell-permeable, highly potent, selective, reversible, and ATP-competitive inhibitor of GSK-3α/β (IC_{50} = 5 nM).	1 mg	92
GSK-3 Inhibitor X	361551	An acetoxime analog of BIO, GSK-3 Inhibitor IX (Cat. No. 361550) that exhibits greater selectivity for GSK-3α/β (IC_{50} = 10 nM) over Cdk5/p25, Cdk2/A and Cdk1/B (IC_{50} = 2.4 µM, 4.3 µM and 63 µM, respectively).	1 mg	92
GSK-3 Inhibitor XIII	361555	An aminopyrazole compound that acts as a potent and ATP-binding site inhibitor of GSK-3 with a K_i of 24 nM.	1 mg 5 mg	65 195
GSK-3 Inhibitor XIV, Control, MeBIO	361556	A cell-permeable N-methylated analog of GSK-3 Inhibitor IX, BIO (Cat. No. 361550). Serves as an inactive control (IC_{50} > 92 µM for Cdk1/B, and > 100 µM for Cdk5/p25 and GSK-3α/β).	1 mg	90
GSK-3β Inhibitor XII, TWS119	361554	A cell-permeable, potent inhibitor of GSK-3β (IC_{50} = 30 nM) that binds to GSK-3β with high-affinity (K_d = 126 nM) and increases the level of β-catenin, a downstream substrate of GSK-3β in the Wnt signaling pathway.	1 mg 5 mg	87 270

NEW

Ready to Use GSK-3 Inhibitor Solutions

InSolution™ GSK-3 Inhibitor IX

A 10 mM (500 µg/140 µl) solution of GSK-3 Inhibitor IX (Cat. No. 361550) in DMSO.

Cat. No. 361552 **500 µg** **$ 61**

InSolution™ GSK-3b Inhibitor VIII

A 25 mM (5 mg/649 µl) solution of GSK-3β Inhibitor VIII (Cat. No. 361549) in DMSO

Cat. No. 361557 **5 mg** **$ 82**

Looking for a Wnt Agonist ?

Wnt Agonist

(2-Amino-4-(3,4-(methylenedioxy)benzylamino)-6-(3-methoxyphenyl)pyrimidine)

A cell-permeable pyrimidine compound that acts as a potent and selective activator of Wnt signaling. Does not inhibit GSK-3β activity (IC_{50} > 60 µM). Mimic the effect of Wnt and induce β-catenin and TCF (T-cell fate)-dependent transcriptional activity (EC_{50} = 700 nM in HEK-293T cells). *Purity: ≥95% by HPLC.*

Cat. No. 681665 **5 mg** **$ 140**

Ref.: Liu, J., et al. 2005. *Angew. Chem. Int. Ed.* **44,** 1987.

Antibodies for Glycogen Synthase Kinase-3

Anti-Glycogen Synthase Kinase 3β, C-Terminal (334-348) Rabbit pAb

Immunoaffinity purified, provided at 1 mg/ml. Immunogen used was a synthetic peptide corresponding to amino acids 334-348 of rat GSK-3β, kinase subdomain XI region coupled to KLH. Recognizes the ~47 kDa in a variety of rat and mouse tissues as well as in human thymus and HeLa cells. Reacts with human, mouse, and rat. Suitable for ELISA, immunoblotting (1 to 4 µg/ml), and immunoprecipitation (12.5 µg/ml).

Cat. No. 361528 **100 µg** **$ 283**

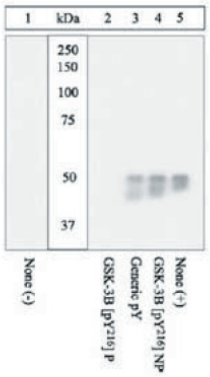
Anti-Glycogen Synthase Kinase-3α/β Mouse mAb (1H8)

Immunoaffinity purified, provided at 1 mg/ml. Immunogen used was recombinant *Xenopus laevis* GSK-3β. Recognizes the ~51 kDa GSK-3α and ~47 kDa GSK-3β protein. Reacts with bovine, canine, hamster, human, mouse, ovine, porcine, rabbit, and rat. Suitable for immunoblotting (1 µg/ml).

Cat. No. 368662 **100 µg** **$ 283**

PhosphoDetect™ Anti-Glycogen Synthase Kinase-3α/β (pTyr$^{279/216}$) Rabbit pAb

Immunoaffinity purified. Immunogen used was a synthetic peptide surrounding the Tyr$^{279/216}$ phosphorylation sites of human GSK-3α/β. Recognizes the ~51 kDa GSK-3α protein phosphorylated at Tyr^{279} and the ~47 kDa protein GSK-3β phosphorylated at Tyr^{216}. Reacts with human, mouse, and rat. Suitable for ELISA, immunoblotting (1:1000), and immunocytochemistry (1:400).

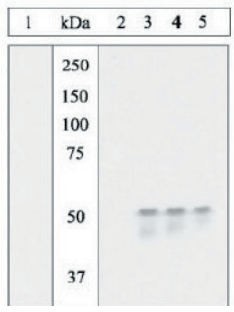


Detection of human GSK-3β phosphorylated on Tyr^{216} by immunoblotting. Samples:Cell lysates from control cells (lane 1) or control lysates containing human recombinant GSK-3β (lanes 2-5). Primary antibody: PhosphoDetect™ Anti-Glycogen Synthase Kinase-3α/β (pTyr$^{279/216}$) Rabbit pAb (Cat. No. ST1013) (1:1000) pre-incubated without peptide (lanes 1, 5), with phosphopeptide immunogen (lane 2), a generic phosphoserine-containing peptide (lane 3), and non-phosphopeptide corresponding to the immunogen (lane 4). Detection: chemiluminescence.

Cat. No. ST1013 **10 T** **$ 295**

PhosphoDetect™ Anti-Glycogen Synthase Kinase-3β (pSer^{9}) Rabbit pAb

Immunogen used was a synthetic phosphopeptide surrounding the Ser^{9} phosphorylation site of human GSK-3β. Recognizes the ~47 kDa GSK-3β phosphorylated on Ser^{9} in insulin or IGF-1 treated, serum-starved 3T3-L1 cells. Reacts with human and rat. Suitable for immunoblotting (1:1000).



Detection of human GSK-3β, phosphorylated on Ser^{9}, by immunoblotting. Samples: Control lysate (lane 1) or control lysated spiked with recombinant GSK-3β (lanes 2-5). Primary antibody: PhosphoDetect™ Anti-Glycogen Synthase Kinase-3β (pSer^{9}) Rabbit pAb (Cat. No. {PS1018) (1:1000) preincubated without peptide (lanes 1,5) or with phosphopeptide immunogen (lane 2), non-phosphopeptide corresponding to the immunogen (lane 3), or a generic phosphoserine-containing peptide(lane 4). Detection: chemiluminescence.

Cat. No. PS1018 **10 T** **$ 295**

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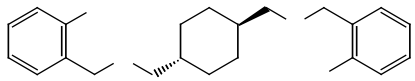
Looking for Inhibitors of Sonic Hedgehog Signaling?

AY 9944

A cell-permeable inhibitor of 7-dehydrocholesterol reduc-



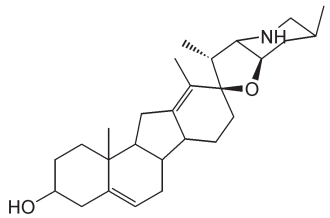
tase (Δ^7-sterol reductase) (IC_{50}= 13 nM). Does not interrupt Shh signaling in ovo, but it blocks the response to Shh-N in explants cultured without an exogenous cholesterol source. *Purity: ≥95% by HPLC.* M.W. 464.3

Cat. No. 190080 **5 mg** **$ 112**

Ref.: Yamashita, Y., et al. 2005. *Endocrinology* **146,** 186; Incardona, J.P., et al. 1998. *Development* **125,** 3553; Moebius, F.F., et al. 1998. *Proc. Natl. Acad. Sci. USA* **95,** 1899. Yoshida, Y. 1985. *J. Biochem.* **98,** 1669.

Cyclopamine, *V. californicum*

A steroidal alkaloid and cholesterol mimic that disrupts cholesterol bio-synthesis and specifically antagonizes Shh signaling through direct interaction with Smo (smoothened). *Purity: ≥95% by HPLC.* M.W. 411.6.



Cat. No. 239803 **1 mg** **$ 117**

Ref.: Berman, D.M., et al. 2003. *Nature,* **425,** 846; Thayer, S.P., et al. 2003. *Nature,* **425,** 851; Watkins, D.N., et al. 2003. *Nature* **422,** 313; Chen, J.K., et al. 2002. *Proc. Natl. Acad. Sci. USA* **99,** 14071; Taipale, J., et al. 2000. *Nature* **406,** 1005.

Cyclopamine-KAAD

A potent, cell-permeable analog of Cyclopamine (Cat. No. 239803) that specifically inhibits the Hedgehog (Hh) signaling with similar or lower toxicity (IC_{50} = 20 nM in Shh-LIGHT2 assay; 50 nM in $p2^{Ptch-/-}$cells; 500 nM in SmoA1-LIGHT cells). Binds to SmoA1 and promotes its exit from the endoplasmic reticulum. Suppresses both the ShhNp-induced pathway activity and SmoA1-induced reporter activity. *Purity: ≥95% by TLC.* M.W. 698.0.

Cat. No. 239804 **100 µg** **$ 128**

Ref.: Ma, X., et al. 2005. *Carcinogenesis* **26,** 1698.

Ref.: Watkins, D.N., et al. 2003. *Nature* **422,** 313. Berman, D.M., et al. 2002. *Science* **297,** 1559. Chen, J.K., et al. 2002. *Proc. Natl. Acad. Sci. USA* **99,** 14071. Chen, J.K., et al. 2002. *Genes Dev.* **16,** 2743. Frank-Kamenetsky, M., et al. 2002. *J. Biol.* **1,** 10. Taipale, J., et al. 2000. *Nature* **406,** 1005.

Jervine

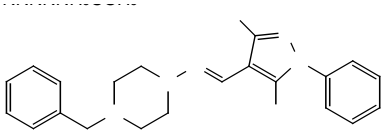
A cell-permeable steroidal alkaloid similar to cyclopamine (Cat. No. 239803) that displays teratogenic effects and induces cyclopia by blocking Shh signaling (IC_{50} ~ 500 - 700 nM in s12 cells). *Purity: ≥98% by TLC.* M.W. 425.6

Cat. No. 420210 **1 mg** **$ 92**

Ref.: Williams, J.A., et al. 2003. *Proc. Natl. Acad. Sci. USA* **100,** 4616; Cooper, M. K., et al. 2003. *Nat. Genet.* **33,** 508; Mistretta, C.M., 2003. *Dev. Biol.* **254,** 1.

SANT-1

A potent antagonist of Shh signaling (IC_{50} = 20 nM in the Shh-LIGHT2 assay and in $Ptch1^{-/-}$ cells) that acts by binding directly to Smoothened (Smo; K_d = 1.2 nM). Unlike Cyclopamine (Cat. No. 239803), SANT-1 equipotently inhibits the activities of both wild-type and oncogenic Smo (IC_{50} = 30 nM in SmoA1-LIGHT2 assay).



Cat. No. 559303 **5 mg** **$ 106**

Ref.: Chen, J.K., et al. 2002. *Proc. Natl. Acad. Sci. USA* **99,** 14071; Frank-Kamenetsky, M., et al. 2002. *J. Biol.* **1,** 10.

Tomatidine, HCl

A steroidal alkaloid that structurally resembles Cyclopamine (Cat. No. 239803), but lacks the capacity to inhibit Shh signaling.

Cat. No. 614350 **25 mg** **$ 46**

NEW Casein Kinase Inhibitors

Product	Cat. No.	Comments	Size	Price
Casein Kinase I Inhibitor, D4476	218696	A cell-permeable, potent, relatively specific ATP-competitive inhibitor of CK1 (IC_{50} = 200 nM from *S. pombe*; 300 nM for CK1δ) and ALK5 (IC_{50} = 500 nM).	1mg	117
InSolution™ Casein Kinase I Inhibitor, D4476	218705	A 10 mM (1 mg/251 µl) solution of Casein Kinase I Inhibitor, D4476 (Cat. No. 218696) in DMSO.	1 mg	117
Casein Kinase II Inhibitor I (4,5,6,7-Tetrabromo-benzotriazole)	218697	A cell-permeable, highly selective, ATP/GTP-competitive inhibitor of CK2 (IC_{50} = 900 nM and 1.6 µM, using rat liver and human recombinant CK2, respectively) and DYRK (IC_{50} < 1 µM for DYRK1a).	10 mg	71
InSolution™ Casein Kinase II Inhibitor I	218708	A 10 mM (5 mg/1.15 ml) solution of Casein Kinase II Inhibitor I (Cat. No. 218697) in DMSO.	5 mg	51
Casein Kinase II Inhibitor II, DMAT	218699	A cell-permeable, potent, high affinity and ATP-competitive inhibitor of CK2 (IC_{50} = 140 nM rat liver; K_i = 40 nM). Displays ~ 1,300 fold greater selectivity over CK1 (IC_{50} > 200 µM) and is superior to CK2 inhibitor, TBB (Cat. No. 218697).	5 mg	87
InSolution™ Casein Kinase II Inhibitor, DMAT	218706	A 10 mM (5 mg/1.05 ml) solution of Casein Kinase II Inhibitor, DMAT (Cat. No. 218699), in DMSO	5 mg	87

Check Point kinases: The Active Gatekeepers of Cell Cycling

Checkpoint kinases, Chk1 and Chk2, participate in various DNA-damage responses, including cell-cycle checkpoints, genome maintenance, DNA repair and apoptosis. They phosphorylate several key proteins involved in cell cycle and block their activity. Chk1 is activated by phosphorylation of Ser^{317} and Ser^{345} in response to DNA damage. Activated Chk1 phosphorylates Ser^{123} of Cdc25A, which targets it for ubiquitin-mediated degradation. The phosphorylated Cdc25A cannot dephosphorylate and activate Cdk1 and Cdk2, which results in arrest of cell cycle in the G1, S and G2 phases. Chk1 is an ideal chemosensitization target and its inhibition can sensitize tumors, particularly those with p53-deficiency, to various chemotherapeutic agents. Chk2 is activated by DNA-strand-breaking agents, such as ionizing radiation and topoisomerase inhibitors through the ATM-dependent pathway. The role of Chk2 in checkpoints is not clearly understood. However, it is reported to phosphorylate Cdc25A and inhibit its activity. Chk2 also phosphorylates Ser^{20} at the amino-terminal activation domain of p53 and regulates levels of p53 in response to DNA double strand breaks. Inhibitors of Chk1 and Chk2 have shown potential to enhance the efficacy of DNA-damaging cancer therapeutic agents by selectively increasing the sensitivity of tumor cells.

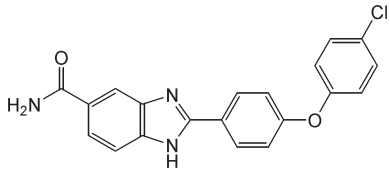
Ref.: Sorenson, C.S., et al. 2005. *Nat. Cell Biol.* **7**, 195; Zhou, B.B.S. and Bartek, J. 2004. *Nat. Rev. Cancer* **4**, 216; Craig, A., et al. 2003. *EMBO Rep.* **4**, 787; O'neill, T., et al. 2002. *J. Biol. Chem.* **277**, 16102; Zhao, H., et al. 2002. *Proc. Natl. Acad. Sci. USA* **99**, 14795; Graves, P.R., et al. 2000. *J. Biol. Chem.* **275**, 5600.

NEW Check Kinase Inhibitors

Chk2 Inhibitor II

(2-(4-(4-Chlorophenoxy)phenyl) -1H-benzimidazole-5-carboxamide)

A cell-permeable, potent, ATP-competitive inhibitor of Chk2 (IC_{50} = 15 nM). Displays ~ 1,000-fold greater selectivity over Cdk1/B and CK1 (IC_{50} = 12 µM and 17 µM, respectively) and only weakly affects the activities of a panel of 31 kinases (< 25% inhibition at 10 µM), including Chk1. *Purity: ≥95% by HPLC.*



Cat. No. 220486 **1 mg** **$ 90**

Ref.: Arienti, K. L., et al. 2005. *J. Med. Chem.* **48**, 1873.

Debromohymenialdisine, *Stylotella aurantium*

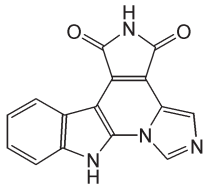
A highly selective inhibitor of G_2 phase DNA damage checkpoint (IC_{50} = 8 µM). Acts by blocking the activities of checkpoint kinases Chk1 (IC_{50} = 3 µM) and Chk2 (IC_{50} = 3.5 µM). Inhibition is competitive with respect to ATP. *Purity: ≥95% by HPLC.*

Cat. No. 252010 **100 µg** **$ 95**

Ref.: Curman, D., et al. 2001. *J. Biol. Chem.* **276**, 17914.

Isogranulatimide

A cell-permeable alkaloid containing indole/maleimide/imidazole skeleton that acts as a potent and ATP-competitive inhibitor of Chk1 (IC_{50} = 100 nM) and GSK-3β (IC_{50} = 500 nM). Displays selectivity for Chk1 over Chk2, Cdk1, and DNA-PK (IC_{50} = 3 µM, 4 µM, and 10 µM, respectively). *Purity: ≥95% by HPLC.* M.W. 276.3.

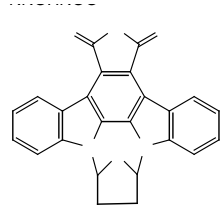


Cat. No. 371957 **1 mg** **$ 135**

Ref.: Jiang, X., et al. 2004. *Mol. Cancer Ther.* **3,** 1221. Roberge, M., et al. 1998. *Cancer Res.* **58,** 5701.

SB 218078

A potent and selective inhibitor of Chk1 *in vitro*. Shown to blocks Chk1 phosphorylation of Cdc25C (IC_{50} = 15 nM). Exhibits only a weak effect on Cdc2 (IC_{50} = 250 nM) and



PKC (IC_{50} = 1 µM). *Purity: ≥90% by HPLC.* M.W. 393.4.

Cat. No. 559402 **1 mg** **$ 103**

Ref.: Zhao, B., et al. 2002. *J. Biol. Chem.* **277,** 46609. Jackson, J.R., et al. 2000. *Cancer Res.* **60,** 566

Purified Check Kinase...

Chk1, His•Tag®, Human, Recombinant

The full length Chk1 expressed in insect cells. *Specific activity: ≥500 units/mg protein. One unit is defined as the amount of enzyme that will transfer 1.0 nmol of phosphate to Chk1 substrate (KKKVSRSGLYRSPSMPENLNRPR) per minute at 30°C, pH 7.5 Purity: ≥90% by SDS-PAGE.* M.W. 56,000

Cat. No. 220479 **10 µg** **$ 324**

Ref.: Heffernan, T.P., et al. 2002. *Mol. Cell. Biol.* 22, 8552; Zhao, H., and Piwnica-Worms, H. 2001. *Mol. Cell. Biol.* 21, 4129; Hutchins, J.R., et al. 2000. *FEBS Lett.* **466,** 91.

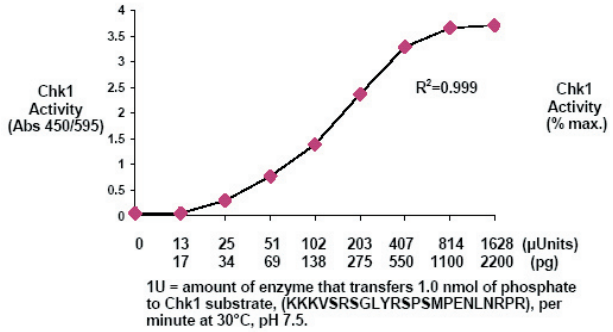
Antibodies for Check Point Kinases

Product	Cat. No.	Comments	Size	Price
PhosphoDetect™ Anti-Chk1 (pSer^{317}) Rabbit pAb	DR1025	Immunoaffinity purified. Recognizes the ~56 kDa Chk1 protein when phosphorylated on Ser^{317}. Reacts with human, monkey, mouse, and rat. **IB, IC, PS.**	50 µl	168
PhosphoDetect™ Anti-Chk2 (pThr^{68}) Rabbit pAb	DR1026	Protein A and immunoaffiinity purified. Recognizes the ~62-66 kDa Chk2 protein phosphorylated on Thr^{68}. Reacts with human and monkey. **FC, IB, IC, IP.**	50 µl	168
Anti-Chk2 (Ab-2) Mouse mAb (2CHK01)	CC44	Protein G purified (200 µg/ml). Recognizes the ~62-70 kDa human Chk2 protein. Does not cross-react with Chk1. Reacts with human. **IB, IP.**	100 µg	238
Anti-Chk1 (1-474) Sheep pAb	PC423	Purified (1 mg/ml). Recognizes the ~54 kDa Chk1 protein. Reacts with human. **IB.**	250 µg	238
Anti-Chk2 Sheep pAb	220540	Immunoaffinity purified (800 µg/ml) Recognizes the ~62-66 kDa Chk2 protein. Reacts with human. **IB, IP.**	50 µg	169
Anti-Chk2/hCds1 (Ab-1) (526-541) Rabbit pAb	PC483	Purified (100 mg/ml). Recognizes the ~62-66 kDa Chk2 protein. Reacts with human and mouse. **IF, PS.**	100 µg	224

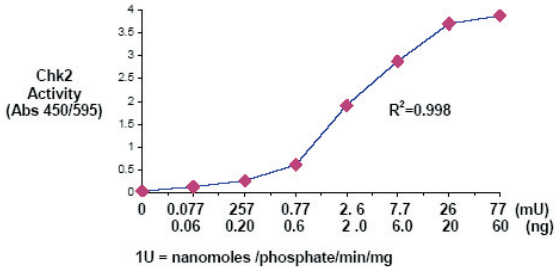
K-LISA™ Checkpoint Activity Kit

A rapid, sensitive, 96-well ELISA-based activity assay kit suitable for measuring the kinase activity of purified or partially purified Chk1 and Chk2 preparations, *in vitro* Chk1 and Chk2 inhibitor screening, and for assessing the regulation of Chk1 and Chk2 in cell signaling. The assay utilizes a biotinylated peptide substrate (KKKVSRSGLYRSPSMPENLNRPR) that is phosphorylated on the third serine by Chk1 and Chk2. The phosphorylated substrate is detected with a phosphoserine detection antibody, followed by the HRP-antibody conjugate and color development with TMB Substrate. Addition of inhibitor (Staurosporine; Cat. No. 569397) serves as a negative control.

Cat. No. CBA020 **1 kit** **$ 365**



Activity of purified Chk1. The activity of His-Tag® Human Recombinant Chk1 (Cat. No. 220479) was determined using protocol A described in the user protocol. Assay range: 34 to 1100 pg (740 units/mg).

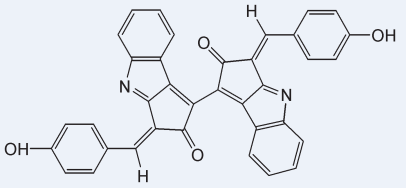


Activity of purified Chk2. The activity of Human recombinant Chk2 was determined using Protocol A described in the user protocol. Assay range: 200 pg to 20 ng (1283 units/mg).

NEW Polo-Like Kinase Inhibitor

Scytonemin, *Lyngbya* sp.

A cell-permeable, selective, reversible, non-toxic, and mixed type inhibitor of *polo*-like kinase 1 (Plk1; IC_{50} = 2.0 µM) and PKCβI (IC_{50} = 3.4 µM). Also shown to inhibit PKCβII (IC50 = 2.7 µM)and several other cell cycle regulatory kinases (IC_{50} = 1.2 µM, 1.4 µM, and 3.0 µM for Myt1, Chk1, and Cdk1/B). *Purity: ≥85% by HPLC.*

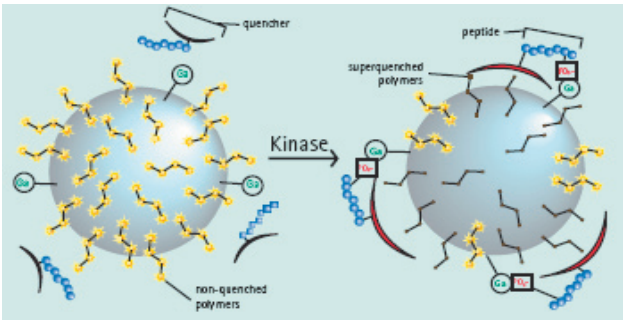


Cat. No. 565715 **1 mg** **$ 95**

Ref.: Stevenson, C.S., et al. 2002. *J. Pharmacol. Exp. Ther.* **303**, 858; Stevenson, C.S., et al. 2002. *Inflamm. Res.* **51**, 112.

Looking for High Sensitivity and Speed in your Protein Kinase Assays?... Try our TruLight™ Kinase Assay Kits

Our TruLight™ Protein Kinase Assay Kits exploit the phenomenon of superquenching and provide greatly enhanced sensitivity. These kits utilize a highly specialized sensor that consists of a fluorescent polymer coated onto microspheres co-localized with phosphate coordinating gallium ions (Ga^{3+}). The non-phosphorylated peptide substrate, specific for each protein kinase, is covalently linked to a superquenching molecule. Following the phosphorylation of serine, threonine, or tyrosine residues on the peptide substrate, the phophorylated peptide binds to the sensor resulting in superquenching of polymer fluorescence. The resulting decrease in fluorescence can then be used as a measure of protein kinase activity.



The main features of these kits are:

- Easy to use; homogeneous one-step "mix and measure" process
- Provided in a 96-well format
- Can be used with either peptide or protein substrates
- Excitation max: ~ 450 nm; emission max: ~ 490 nm
- Can be used with recombinant or purified protein kinases
- Excellent sensitivity and greater signal stability.
- Excellent Z′ values even at 10% conversion level
- Greater ATP tolerance (50 to 100 µM)
- Can be used for screening protein kinase inhibitors and activators

Cat. No.	Product	Sensitivity	Size	Price
539705	TruLight™ Akt1/PKBα Kinase Assay Kit	25 pM	1 kit	515
539716	TruLight™ Aurora A Kinase Assay Kit	6.9 pM	1 kit	515
539715	TruLight™ ERK1/2 Assay Kit	ERK1 = 25 pM ERK2 = 66 pM	1 kit	515
539712	TruLight™ PKA Assay Kit	1.2 pM	1 kit	515
539707	TruLight™ PKC_{α} Assay Kit	2.5 pM	1 kit	515
539713	TruLight™ $PKC_{\beta I/II}$ Assay Kit	$PKC_{\beta I}$ = 4.2 pM $PKC_{\beta II}$ = 2.2 pM	1 kit	515
539708	TruLight™ PKC_{ε} Assay Kit	7.6 pM	1 kit	515
539709	TruLight™ RSK-2 Assay Kit	75 pM	1 kit	515
539706	TruLight™ Src Kinase Assay Kit	31.6 pM	1 kit	515
539710	TruLight™ p38α Kinase Assay Kit	211 pM	1 kit	515
539711	TruLight™ p70S6 Kinase Assay Kit	800 pM	1 kit	515

Also Available...

TruLight™ Universal Kinase/Phosphatase Assay Kit

A fluorescence superquenching-based assay for rapid and sensitive assay of protein kinase or protein phosphatase of choice. Protein kinase activity is measured as a decrease in fluorescence resulting from the superquenching effect. The protein phosphatase activity reverses the superquenching effect by removing the phosphate group from the peptide substrate, and is measured as an increase in fluorescence.

Cat. No. 539714 **1 kit** **$ 515**

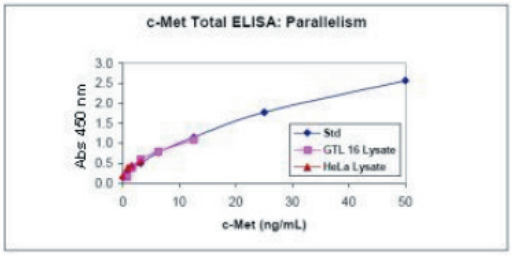
Other NEW Protein Kinase Inhibitors

Product	Cat. No.	Comments	Size	Price
AMPK Inhibitor, Compound C	171260	A cell-permeable potent, selective, reversible, and ATP-competitive inhibitor of AMPK (AMP-activated protein kinase; K_i = 109 nM in the presence of 5 µM ATP and the absence of AMP).	1 mg 5 mg	70 235
InSolution™ AMPK Inhibitor, Compound C	171261	A 10 mM (1 mg/100 µl) solution of AMPK Inhibitor, Compound C (Cat. No. 171260) in DMSO.	1 mg	70
Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	124018	A cell-permeable, potent, selective inhibitor of Akt1/Akt2 activity (IC_{50} = 58 nM, 210 nM). The inhibition appears to be pleckstrin homology (PH) domain-dependent.	1 mg	130
Cdk1/2 Inhibitor III	217714	A cell-permeable, highly potent, ATP-competitive inhibitor of Cdk1/cyclin B and Cdk2/cyclin A (IC_{50} = 600 pM and 500 pM, respectively).	1 mg	85
ERK Inhibitor (3-(2-Aminoethyl)-5-((4-ethoxyphenyl)methylene) -2,4-thiazolidinedione, HCl)	328006	A cell-permeable anti-proliferative agent ($IC_{50} \leq 25$ mM in HeLa, A549, and SUM-159 tumor cells) that preferentially binds to ERK2 (K_d of ~5 mM) and prevents its interaction with protein substrates.	5 mg	85
IGF-1R Inhibitor, PPP (Picropodophyllin)	407247	A cell-permeable, a non-competitive, potent, and specific inhibitor of IGF-1R both *in vitro* (IC_{50} = 1 nM in cell-free kinase assay; ≤ 60 nM for cell viability and receptor autophosphorylation in melanoma cell lines) and *in vivo* (complete inhibition of IGF-1R-dependent tumor cell growth at 20 mg/kg/12 hr i.p. in SCID mice)..	1 mg	92
Indirubin Derivative E804	402081	A cell-permeable indirubin derivative that blocks the Src-Stat3 signaling pathway. Acts as a potent, ATP-competitive inhibitor of Src kinase (IC_{50} = 430 nM), Cdk1/cylin E (IC_{50} = 210 nM), Cdk2/cyclin A (IC_{50} = 540 nM), and Cdk1/cyclin B (IC_{50} = 1.65 µM).	1 mg	85
JNK Inhibitor V (AS601245)	420129	A cell-permeable, potent, selective, and ATP-competitive inhibitor of c-Jun N-terminal kinase (JNK, IC_{50} = 150, 220, and 70 nM for hJNK1, hJNK2, and hJNK3, respectively; 120 nM for rat JNK3).	5 mg	140
JAK2 Inhibitor II (1,2,3,4,5,6-Hexabromocyclohexane)	420132	A cell-permeable inhibitor that interacts with a solvent-accessible pocket near the activation loop of JAK2 and acts as a specific and direct inhibitor of JAK2 autophosphorylation (maximal inhibition at 50 mM in BSC-40 cells over-expressing JAK2).	25 mg	85
Rho Kinase Inhibitor III, Rockout (3-(4-Pyridyl)-1H-indole)	555553	A cell-permeable, selective, ATP-competitive inhibitor of Rho kinase (IC_{50} = 25 µM). Does not inhibit the activation of Rho kinase.	10 mg	85
Tyrene CR4	655230	A cell-permeable, potent inhibitor of JAK2 and Bcr-Abl (IC_{50} ~ 100-600 nM and 500-700 nM, respectively), and displays selectivity over other related kinases, namely, Btk, Lck, Lyn, Src, Syk and ZAP-70 (IC_{50} > 5 mM).	5 mg	80

NEW! ELISA Kit for Measuring Protein Kinase Activities

c-Met ELISA Kit

96-well format; Sensitivity: <4 ng/ml; Assay Range: 0.78-50 ng/ml; Assay Time: 4 h; Sample Type: Cells



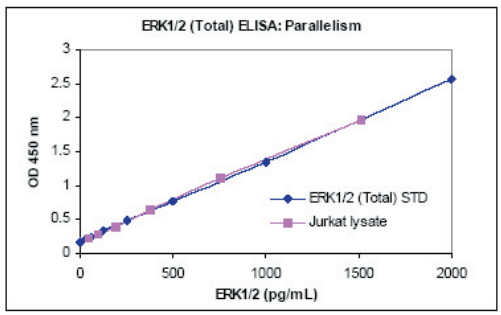
Detects and quantifies the levels of c-Met protein independent of its phosphorylation state. This kit is designed for use with human samples.

Natural c-Met from GTL and HeLa cell lysates was serially diluted in standard diluent buffer. Absorbance at each dilution was plotted against the c-Met standard curve.

Cat. No. CBA031 **1 kit** **$ 575**

ERK1/2 ELISA Kit

96-well format; Sensitivity: < 16 pg/ml; Assay Range: 31.2-2000 pg/ml; Assay Time: 4 h; Sample Type: Cells Detects and quantifies the level of ERK 1/2 proteins independent of their phosphorylation state. Although, this kit is designed for use with human cell lines, platelets, and lymphocytes, it cross-reacts with mouse and rat cells.



Natural ERK1/2 from human Jurkat cell lysates were serially diluted in standard diluent buffer. Absorbance at each dilution was plotted against the standard ERK1/2 standard curve.

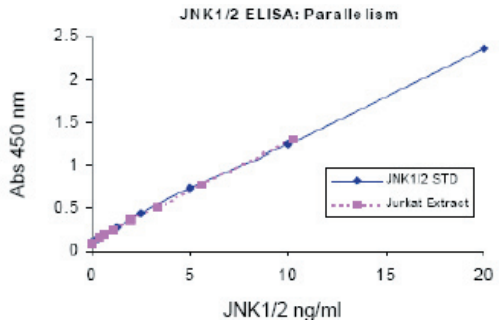
Cat. No. CBA032 **1 kit** **$ 575**

Technical Support
Phone 800 628 8470
E-mail calbiochem@emdbiosciences.com

JNK 1/2 ELISA Kit

96-well format; Assay Range: 0.31-20 ng/ml; Assay Time: 4 h; Sample Type: Cells

Detects and quantifies the level of JNK1 and JNK2 proteins independent of their phosphorylation state. This kit can be used for detecting and quantifying JNK1/2 proteins found in human and mouse samples.



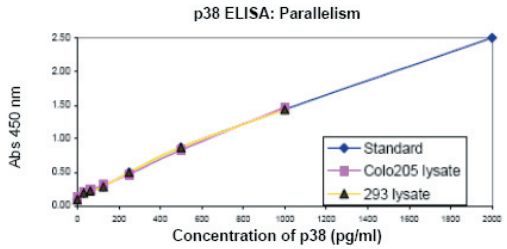
Natural JNK1/2 from Jurkat cells was extracted in 6M urea and serially diluted in standard diluent buffer. Absorbance at each dilution was plotted against the JNK1/2 standard curve.

Cat. No. CBA033 **1 kit** **$ 575**

p38 MAP Kinase ELISA Kit

96-well plate; Sensitivity: ≤16 pg/ml; Assay Range: 31.2-2000 pg/m; Assay Time: 4 h; Sample Type: Cells

Detects and quantifies the levels of p38 MAPK protein independent of its phosphorylation state. p38 MAPK activation occurs in response to a variety of extracellular stimuli including osmotic shock, inflammatory cytokines, LPS, and UV light. Although this kit is designed for human samples, it cross-reacts with mouse and monkey.



Natural p38 MAPK from human colo205 and 203 cell lysates were serially diluted in standard diluent buffer. Absorbance at each dilution was plotted against the p38 MAPK standard curve.

Cat. No. CBA029 **1 kit** **$ 575**

Heat Shock Proteins: Protecting the Cancer Cells

Depending on the level of stress, injured cells may undergo either necrosis or apoptosis. Under extreme stress conditions, when there is diminution in regulated activation of apoptotic pathways, cells undergo necrosis. At lower stress levels, cells activate their apoptotic machinery. However, at sub-lethal stress levels, cells may attempt to survive and activate a stress response system that includes a rapid induction of heat shock proteins (Hsp). Hsp interact with diverse protein substrates and assist in their folding and in the elimination of any misfolded or damaged molecules. They are transiently expressed during cell cycle to prevent differentiating cells from undergoing apoptosis. Many tumor cells have constitutively elevated levels of Hsp that impart protection against cytotoxic agents thereby raising the apoptosis threshold of these cells. This abnormal expression of Hsp may lead to multi-drug resistance in aggressively growing tumors. Many Hsp, including Hsp27 and Hsp70, have been shown to block apoptosis. Hsp can also allow cancerous cells to escape the immunosurveillance mediated by death ligands and can render these cells resistant to chemotherapy. Hence, Hsp are fast becoming new targets for therapeutic interventions.

Ref.: Chiosis, G., et al. 2004. *Drug Discov. Today* **9,** 881; Odunuga, O.O., et al. 2004. *Bioessays* **26,** 1058; Creagh, E.M., et al. 2000. *Leukemia* **14,** 1161; Jolly,C., and Morimoto, R.I. 2000. *J. Natl. Cancer Inst.* **92,** 1564; Gibbons, N.B., et al. 2000. *Prostate* **45,** 58; Arrigo, A.P. 2000. *Pathol. Biol. (Paris)* **48,** 280.

NEW Antibodies for Heat Shock Proteins

Product	Cat. No.	Comments	Size	Price
Anti-GroEL, Mouse Mab	CB1007	Monoclonal IgG_1, purified. Clone 9A1/2. Immunogen used was GroEL purified from an over-producing strain of *E. coli*. Detects the ~60 kDa bacterial chaperonin GroEL from *E. coli* and *Pseudomonas*. Does not cross react with eukaryotic Hsp60, a GroEL homolog. **IB, IP**	50 µl	214
Anti-Hsp27, Rabbit PAb	CA1015	Polyclonal IgG, undiluted serum. Immunogen used was recombinant Hsp27 protein containing an N-terminal His·Tag® sequence. Detects the 27 kDa Hsp27 in human. Hsp27 is reported to have altered expression in some cancers. **IB**	50 µl	138
Anti-Hsp90α, Rabbit PAb	CA1016	Polyclonal IgG, undiluted serum. Immunogen used was recombinant Hsp90α protein containing an N-terminal His·Tag® sequence. Detects the 90 kDa Hsp90α in human. Hsp90α is reported to interact with MMP-2 and promote its activation. **IB**	50 µl	138
Anti-Hsp90β, Rabbit PAb	CA1017	Polyclonal IgG, undiluted serum. Immunogen used was recombinant Hsp90β protein containing an N-terminal His·Tag® sequence. Detects the 90 kDa Hsp90β in human. **IB**	50 µl	138

IB: immunoblotting; **IP:** immunoprecipitation; **MAb:** monoclonal antibody; **PAb:** polyclonal antibody

NEW Heat Shock Proteins

Hsp27, His·Tag®, Human, Recombinant

A full-length human Hsp27 (amino acids 1-205) (Target symbol = HSPB1, GenBank accession number = BC00510) with an N-terminal His·Tag® sequence was expressed in and purified from *Spodoptera frugiperda* insect cells. Hsp27 has also been reported to have altered expression in some cancers. It inhibits apoptosis and regulates cytoskeletal dynamics by acting as an actin-binding protein. *Purity: ≥90% by SDS-PAGE.*

Cat. No. 385909 **20 µg** **$ 122**

Ref.: Garrido, C., et al. 2003. *Cell Cycle* **2,** 579; Oesterreich, S., et al. 1993. *Cancer Res.* **53,** 4443; Thor, A., et al. 1991. *J. Natl. Cancer Inst.* **83,** 170.

Hsp90α, His·Tag®, Human, Recombinant

A full-length human Hsp90α (amino acids 1-732) (GenBank target symbol = HSPCA, Accession number = NM_005348) with an N-terminal His·Tag® sequence was expressed in and purified from *Spodoptera frugiperda* insect cells. Native Hsp90α exists primarily as oligomers in the cytoplasm. *Purity: ≥80% by SDS-PAGE.*

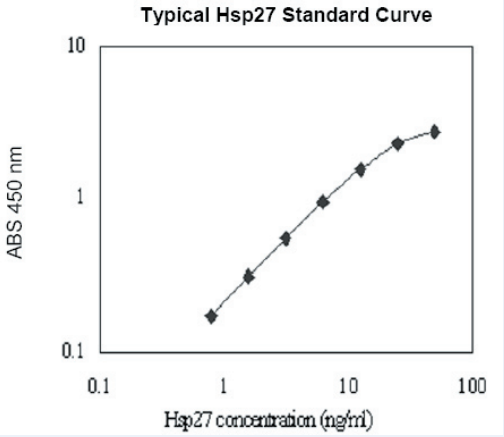
Cat. No. 385901 **20 µg** **$ 128**

Ref.: Zhang, S.L., et al. 1999. *FEBS Lett.* **444,** 130; Nemoto, T., and Sato, N. 1998. *Biochem. J.* **330,** 989; Metz, K., et al. 1996. *FEBS Lett.* **385,** 25; Welch, W.J., and Feramisco, J.R. 1982. *J. Biol. Chem.* **257,** 14949.

Want to Assay Hsp27?...

Hsp27 ELISA Kit

Our 96-well Hsp27 ELISA Kit is designed for the quantitative assay of human Hsp27 in cell lysates, tissue extracts, serum, and plasma. The capture antibody used in this kit is a murine anti-human Hsp27 monoclonal antibody. Standards, samples, and a rabbit polyclonal antibody to human Hsp27 are simultaneously incubated in pre-coated well strips. Following a wash goat anti-rabbit IgG conjugated to HRP is added that binds to the polyclonal human Hsp27. The addition of substrate and subsequent reaction with HRP produces a blue color solution that can be quantified at 450 nm. Assay Range: 0.78-50 ng/ml Assay Time: 3.5 hours.



Hsp27 levels in human plasma, serum, and various cell lines measured using Hsp27 ELISA kit (QIA119)

Plasma:	9-12 ng/ml
Serum:	6- 8 ng/ml
MCF-7 cells:	3.1 ng/µg protein
HeLa cells:	24.2 ng/µg protein
U251 cells:	8.6 ng/µg protein
U373 cells:	4.3 ng/µg protein
HL-60 cells:	0.2 ng/µg protein
SK-BR-3 cells:	6.9 ng/µg protein

Recovery of Recombinant Hsp27 using Hsp27 ELISA Kit

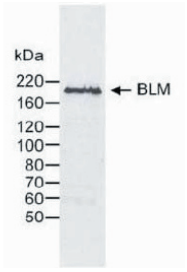
Plasma:	96.5%
Serum:	97.0%
Cell lysates:	96.0%

Cat. No. QIA 119 **1 kit** **$ 464**

Interested in Antibodies for DNA Damage and Repair?

Anti-BLM Rabbit pAb

Immunoaffinity purified (1mg/ml). Immunogen used was a synthetic peptide corresponding to a portion of human Bloom Syndrome (BLM) protein encoded within exon 22 (LocusLink ID 641). Recognizes the ~170 kDa BLM protein. Suitable for immunoblotting (0.1 to 1 µg/ml) and immunoprecipitation (1 to 4 µγ/mg protein from lysate)

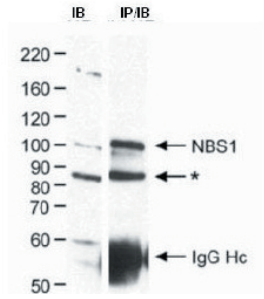


Detection of human BLM by immunoblotting. Sample: Cell lysates (35 µg) from HEK293T cells. Primary antibody: Anti-BLM (Cat. No. DR1034; 0.5 µg/ml). DetectionL chemiluminescence.

Cat. No. DR1034 **50 µg** **$ 138**

Anti-NBS1, Rabbit pAb

Immunoaffinity purified (1 mg/ml). Immunogen used was a synthetic peptide corresponding to a 24 amino acids sequence encoded by exons 2 and 3 of human Nijmegen breakage syndrome 1 (NBS1) (LocusLink ID 4683). Recognizes the ~95 kDa NBS1 protein in nuclear extracts from HeLa cells. Suitable for immunoblotting ((0.1 to 1 µg/ml) and immunoprecipitation (1 to 4 µγ/mg protein from lysate).

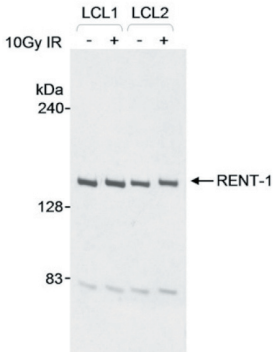


Detection of human NBS1 by immunoblooting and immunoprecipitation. Samples: Nuclear extracts (50 µg for IB and 1 mg for IP) from HeLa cells. Primary antibody: Antib-NBS-1 (Cat. No. DR1033; 0.08 µg/ml for IB and 1.25 µg/ml for IP). Antibody also recognizes a ~85 kDa protein believed to be a proteolytic fragment of NBS-1. Detection: Chemiluminescence

Cat. No. DR1033 **50 µg** **$ 138**

Anti-RENT1 Goat pAb

Immunoaffinity purified (1 mg/ml). Immunogen used was a synthetic peptide corresponding to amino acids at the C-terminus of human RENT1. Recognizes the ~140 kDa RENT1 protein. Suitable for immunoblotting (1:1000 to 2,500).



Detection of human RENT1. Samples: Cell lysates from untreated (-) and irradiated (+) lymphophoid cell lines (LCL1 and LCL2). Primary antibody: Anti-RENT1 Goat pAb (Cat. No. DR1015; 1:1000). Detection: Chemiluminescence.

Cat. No. DR1015 **100 µg** **$ 295**

Anti-SMC5 Rabbit pAb

Immunoaffinity purified (1 mg/ml). Immunogen used was a synthetic peptide corresponding to a sequence encoded within amino acids 1050-1101 (using the numbering given in TrEMBL entry Q8IY18 [GeneID 23137]) of human SMC5. Recognizes the ~130 kDa SMC5 protein in HeLa and HEK 293T cells. Suitable for immunoblotting (0.04 to 0.2 µg/ml) and immunoprecipitation (5 to 10 µg/mg protein from lysate).

Cat. No. DR1030 **50 µg** **$ 138**

Anti-SMC6 Rabbit pAb

Immunoaffinity purified (1 mg/ml). Immunogen used was a synthetic peptide corresponding to a sequence encoded within amino acids 1050-1091 (using the numbering given in TrEMBL entry Q96SB8 [GeneID 79677]) of human SMC6. Recognizes the ~126 kDa SMC6 protein in HeLa and HEK 293T cells. Suitable for immunoblotting (0.04 to 0.2 µg/ml) and immunoprecipitation (5 to 10 µg/mg protein from lysate).

Cat. No. DR1031 **50 µg** **$ 138**

Anti-WRN (400-500) Rabbit pAb

Immunoaffinity purified (1 mg/ml). Immunogen used was a synthetic peptide corresponding to a sequence between amino acids 400-450 of human Werner Syndrome Helicase (WRN) (using the numbering in SwissProt entry Q14191 [GeneID 7486]). Recognizes the ~160 kDa WRN protein in HEK 293T cells. Suitable for immunoblotting (0.04 to 0.2 µg/ml) and immunoprecipitation (2 to 5 µg/mg protein from lysate).

Cat. No. DR1032 **50 µg** **$ 138**

NEW Proteases and Protease Inhibitors

Procathepsin K, Human, Recombinant, *E. coli*

Recombinant human Procathepsin K (amino acids 19-329) expressed in *E. coli*. A member of the papain superfamiliy of cysteine proteinases, synthesized as an inactive proenzyme that is converted to its mature active form by proteolytic cleavage of its 99 amino acid proteptide domain. If the activated enzyme is not used immediately, it is recommended to add methyl methanthiosulfonate (1 mM final concentration MMTS) before storing it. *Purity: ≥95% by SDS-PAGE.* M.W. 35,068

Cat. No. 342001	10 µg	$ 235

HtrA2/Omi, Human, Recombinant, *E. coli*

A mitochondrial trypsin-like serine protease that is pivotal in regulating apoptotic cell death. It is processed to expose an amino-terminal Reaper-like motif similar to SMAC/Diablo. Purity: *Purity: ≥85% by SDS-PAGE.* M.W. 35,900.

Cat. No. 539663	100 µg	$ 315

Ref.: Martins, L., et al. 2002. *J. Biol. Chem.* **277,** 439; Silke, J. and Verhagen, A. 2002. Cell Death. Differ. **9,** 362; Hedge, R. et al. 2002. *J. Biol. Chem.* **277**, 432.

Science **241,** 699;
Sumi, Y., et al. 1989. *J. Biochem.* **106,** 703.

Renin, Human Kidney

Secreted by the juxtaglomerular cells that act on angiotensinogen to produce a decapeptide, angiotensin I, which in turn undergoes cleavage to form angiotensin II. The renin-angiotensin system plays an important role in regulating blood volume, arterial pressure, and cardiac and vascular functions. *Purity: ≥90% by NuPAGE gel.* M.W. 40,000

Cat. No. 553901	150 mU	$ 235

Serpin F2/α2-Antiplasmin, His-Tag®, Human

A member of the Serpin superfamily of the serine protease inhibitors that is responsible for the dissolution of fibrin clots. It is also an efficient inhibitor of trypsin and chymotrypsin. *Purity: ≥95% by SDS-PAGE.* M.W. 67,000.

Cat. No. 539664	10 µg	$ 315

Ref.: Silverman, G.A., et al. 2001. *J. Biol. Chem.* **276,** 33293; Potempa, J., et al. 1988.

NEW Aprotinin Free from Animal products

Aprotinin, Bovine, Recombinant, Nicotiana sp., Animal-Free

A reversible inhibitor of serine proteinases. Contains no animal-derived components. *Specific activity: ≥5.0 TIU/mg protein. Purity: ≥95% by SDS-PAGE.*

Cat. No. 616371	1 mg	$ 50
	5 mg	$ 70
	25 mg	$140

Ask for your free copy today.

Provides information on specificity, solubility, stability, and effectiveness of our well-known protease inhibitor cocktails.

Now Available!

NEW Protease Inhibitor Cocktails with Recombinant Aprotinin

Protease Inhibitor Cocktail Set I, with Animal-Free Aprotinin

Broad range protease inhibitor cocktail. Reconstitute each vial with 1 ml of H_2O to obtain a 100X stock solution. When diluted to 1X, each vial will contain: 500 µM AEBSF, HCl, 150 nM Aprotinin, Recombinant, 1 µM E-64 Protease Inhibitor, 500 µM EDTA, Disodium, and 1 µM Leupeptin, Hemisulfate.

Cat. No. 535142	1 ml	$ 27
	1 set (10 x 1 ml)	$ 196

Protease Inhibitor Cocktail Set III, with Animal-Free Aprotinin

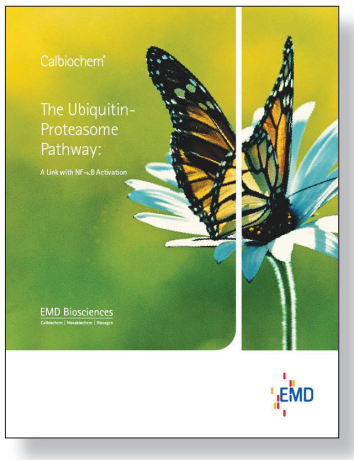
Recommended for use with mammalian cells and tissue extracts and with bacterial cell extracts used for metal chelation chromatography. Each vial contains 100 mM AEBSF, HCl , 80 µM Aprotinin, 5 mM Bestatin , 1.5 mM E-64 Protease Inhibitor, 2 mM Leupeptin, Hemisultate, and 1 mM Pepstatin A. Supplied in 1 ml of DMSO. One ml is sufficient for use with 20 g of tissue.

Cat. No. 535140	1 ml	$ 55
	1 set (5 x 1 ml)	$ 238

Protease Inhibitor Cocktail Set V, with Animal-Free Aprotinin

Protease inhibitor cocktail for serine and cysteine proteases. Reconstitute each vial with 1 ml of H_2O to obtain a 100X stock solution. When diluted to 1X, each vial will contain: 500 µM AEBSF, HCl, 150 nM Aprotinin, Recombinant, 1 µM E-64 Protease Inhibitor, and 1 µM Leupeptin, Hemisulfate.

Cat. No. 535141	1 set (10 x 1 ml)	$ 197

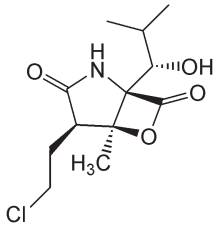


Ask for your free copy today.

Interested in Ubiquitin/Proteasome Research?

Proteasome Inhibitor VII, Antiprotealide

An Omuralide-Salinosporamide hybrid that irreversibly inactivates the β5-subunit of the human 20S proteasome. Shown to be ~2.5-fold more potent than Omuralide (Cat. No. 426102) and somewhat less potent than Salinosporamide A. *Purity: ≥95% by NMR.*. M.W. 275.7.



Cat. No. 539179	50 µg	$ 175

Ref.: Reddy, L.R., et al. 2005. *J. Am. Chem. Soc.* 127, 8974.

Methylated Ubiquitin, Human, Recombinant

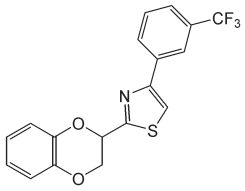
An ubiquitin derivative in which lysine residues are reductively methylated. Methylated ubiquitin can ligate to protein substrates, but does not form polyubiquitin chains. Protein breakdown rates obtained with methylated ubiquitin are slower than those obtained with unmethylated ubiquitin. Useful for reducing the polyubiquitin chain length and for determining rates of ubiquitin conjugation. *Purity: ≥95% by SDS-PAGE.*M.W. 8,500.

Cat. No. 662065	1 mg	$ 92

NEW Cell Death Research Tools...

Caspase Inhibitor X (BI-9B12)

A benzodioxane containing 2,4-disubstituted thiazolo compound that acts as a selective, reversible and competitive inhibitor of caspases (K_i = 4.3 µM, 6.2 µM and 2.7 µM for caspase-3, -7 and -8, respectively). The benzodioxane moiety is shown to fit in the 'aspartate hole' of the caspases and possibly disrupt caspase-8 assisted cleavage of BID, a proapoptotic protein. *Purity: ≥97% by HPLC.* M.W. 363.4.



Cat. No. 218723 **5 mg** **$ 115**

Ref.: Fattorusso, R., et al. 2005. *J. Med. Chem.* **48,** 1649.

cIAP-1, Human, Recombinant, *E. Coli*

A member of the inhibitor of apoptosis family of proteins that inhibits proteolytic activity of mature caspases by interaction of the BIR domain with the active caspase. *Purity: ≥95% by SDS-PAGE.* M.W. 72,300.

Cat. No. 539661 **50 µg** **$ 305**

Ref.: Herrera, B., et al. FEBS Lett. 520, 93; Deveraux, Q.L. and Reed, J.C. 1999. Genes Dev. 13, 239; Roy, N., et al. 1997. EMBO J. 16, 6914.

Necrostatin-1

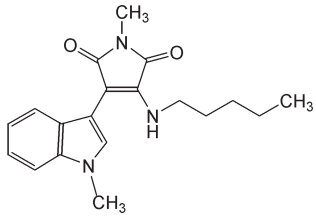
A cell-permeable N-methylated thiohydantoin compound that potently and selectively blocks necroptosis, a non-apoptotic necrotic cell-death pathway, mediated by death-domain receptors (DRs), in vitro (EC_{50} = 494 nM in FADD-deficient Jurkat cells treated with TNF-a). Offers neuroprotection in a mouse model of ischemic brain injury. An inactive control, Nec-1i, is also available (Cat. No. 480066).M.W. 259.3.

Cat. No. 480065 **5 mg** **$ 80**

Ref.: Teng, X., et al. 2005. Bioorg. Med. Chem. Lett.15, (In press); Degterev, A., et al. 2005. Nat. Chem. Biol. 1, 112.

Necrosis Inhibitor, IM-54

A cell-permeable, selective blocker of oxidative stress-induced necrotic cell death (~10 µM in HL60 cells exposed to 100 µM H_2O_2). Does not offer protection against Etoposide (Cat. No.341205) and does not have



any antioxidant properties. *Purity: ≥95% by HPLC.*

Cat. No. 480060 **5 mg** **$ 135**

Ref.: Dodo, K., et al. 2005. *Bioorg. Med. Chem. Lett.* **15,** 3114.

InSolution™ Caspase Inhibitor VI

(Ready to Use Z-VAD-FMK solution)

A 10 mM (1 mg/221 µl) solution of Caspase Inhibitor VI (Cat. No. 219007) in DMSO.

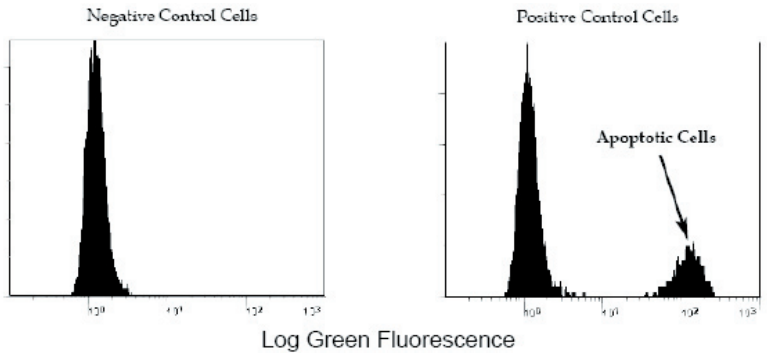
Cat. No. 219011 **1 mg** **$ 177**

NEW Kits for Apoptosis Research

Apo-BrdU™ Kit

A two color TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay for labeling DNA breaks and total cellular DNA to detect apoptotic cells by flow cytometry and microscopy. This kit labels the 3'-hydroxy termini of apoptotic DNA fragments with bromolated deoxyuridine triphosphate nucleotides (Br-dUTP). It is known that Br-dUTP is more readily incorporated into the genome of apoptotic cells compared to larger ligands, such as fluorescein or biotin. Suitable for up to 60 tests. Kit contains: Positive control cells, negative control cells, wash buffer, reaction buffer, TdT, Br-dUTP, rinsing buffer, fluorescein PRB-1 mAb, PI/RNase staining buffer, and a user protocol.

Flow Cytometry data using the Apo-BrdU™ Negative and Positive Control Cells



Cat. No.CBA040 **1 kit** **$ 435**

Apo-Direct™ Kit

A two color TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay for labeling DNA breaks and total cellular DNA to detect apoptotic cells by flow cytometry or laser scanning cytometry. This kit utilizes terminal deoxynucleotidyl transferase (TdT) to catalyze incorporation of fluorescein-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. Suitable for up to 60 tests. Kit contains: Positive control cells, negative control cells, wash buffer, reaction buffer, TdT, rinsing buffer, FITC dUTP, PI/RNase staining buffer, and a user protocol.

Cat. No. CBA041 **1 kit** **$ 435**

CARTOON

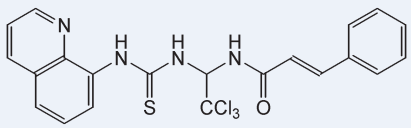
NEW Antibodies for Apoptosis, Cancer, and Cell-Cycle Research

Product	Cat. No.	Comments	Size	Price
Anti-Apollon (4775-4829) Rabbit pAb	AP1031	Immunoaffinity purified (1 mg/ml). Recognizes the ~500 kDa Apollon protein in HeLa cells. Apollon is a membrane-associated inhibitor of apoptosis protein (IAP). Reacts with human. IB (1 µg/ml) and IP (2 µg/mg lysate protein)	50 µg	135
Anti-Androgen Receptor Rat mAb (AN1-15)	CA1022	Purified from ascites fluid (2.4 mg/ml). Detects the ~110 kDa androgen receptor in rat that represents ligand-dependent intracellular protein that stimulates transcription of specific genes by binding to specific DNA sequence following activation by the appropriate hormone. Reacts with human, mouse, non-human primates, and rat. FS (4 µg/ml), IB (1 µg/ml), IC (5 µg/ml), PS (4 µg/ml)	50 µg	280
Anti-Cytochrome c Mouse mAb (6H2.B4)	AP1030	Protein G purified (1 mg/ml). Recognizes the ~15 kDa cytochrome c. Reacts with human, mouse, and rat. FC, IC (3 µg/ml), IP (5 µg/500 µg protein)	50 µg	135
Anti-Cytochrome c Mouse mAb (7H8.2C12)	AP1029	Protein G purified (1 mg/ml). Recognizes the ~15 kDa cytochrome c. Epitope lies between amino acids 93 and 104. Reacts with horse, human, mouse, and rat. FC, IB (1 µg/ml), IC (3 µg/ml).	50 µg	135
Anti-TSC1 (Tuberous Sclerosis 1) Rabbit pAb	AP1032	Immunoaffinity purified (1 mg/ml). Detects the ~120 kDa tuberous sclerosis 1 protein (TSC1) protein in HeLa, MEF, and U2OS cells. Reacts with human and mouse. IB (0.2 µg/ml), IP (1 µg/mg lysate protein).	50 µg	135
PhosphoDetect™ Anti-mTOR (pSer2448) Rabbit pAb	PS1020	Immunoaffinity purified (1 mg/ml). Detects ~290 kDa mTOR protein phosphorylated on Ser2448 in EGF-treated HEK293 cells. Reacts with human. IB (3 µg/ml).	50 µg	135
Anti-PARC/H7-AP1 (900-950) Rabbit pAb	DR1028	Peptide affinity purified (1 mg/ml). Recognizes the ~270 kDa PARC/H7-AP1 protein in U2OS cells. PARC serves as a cytoplasmic anchor for p53-associated protein complexes. Reacts with human. IB (1:1000 –5000), IP (2 to 5 µg/mg lysate).	100 µg	281

FC: Flow cytometry; **FS:** Frozen sections; **IB:** immunoblotting; **IC:** immunocytochemistry; **IP:** immunoprecipitation; **MAb:** monoclonal antibody; **PAb:** polyclonal antibody

eIF-2a Inhibitor, Salubrinal

A cell-permeable, selective inhibitor of eukaryotic translation initiation factor 2-subunit α (eIF-2α) dephosphorylation by phosphatase complexes. Shown to offer protection against ER stress-mediated apoptosis (EC_{50} ~ 15 µM in PC12 cells stimulated with 750 ng/ml of Tunicamycin (Cat. No. 654380). Shown to block HSV replication in both HSV-Vero cells (IC_{50} ~ 3 µM for reducing plaque formation) and mouse cornea infection model by inhibiting eIF-2α dephosphorylation. *Purity: ≥90% by HPLC.*



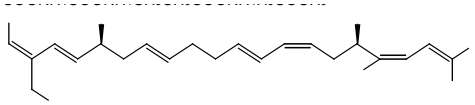
Cat. No. 324895 **5 mg** **$ 125**

Ref.: Boyce, M., et al. 2005. *Science* **307**, 935.

Tools for Mitochondrial Metabolism Research

Bongkrekic Acid, Triammonium Salt

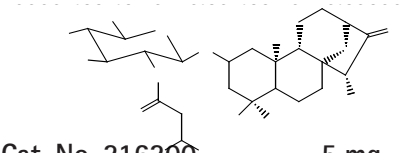
A potent inhibitor of the mitochondrial megachannel (permeability transition pore). Significantly reduces signs of apoptosis induced by nitric oxide. Prevents the apoptotic breakdown of the inner mitochondrial transmembrane potential ($\Delta\psi m$).



Cat. No. 203671 **500 µg** **$ 257**

Carboxyatractyloside, *Atractylis gummifera*

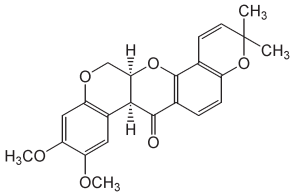
A highly selective inhibitor of the mitochondrial ADP/ATP carrier (AAC; Ki <10 nM).



Cat. No. 216200 **5 mg** **$ 201**

Deguelin, *Mundulea sericea*

A cell-permeable, potent inhibitor of mitochondrial bioenergetics (IC_{50} = 6.9 nM for NADH:ubiquinone oxidoreductase activity in bovine heart ETP; IC_{50} = 11 nM for phorbol ester induced ornithine decarboxylase activity in MCF-7 cells. Induces apoptosis and selectively blocks Akt activation with minimal effects on MAPK signaling. Reported to activate AMPK activity.



Cat. No. 252740 **5 mg** **$ 65**

Ref.: Gills, J.J., et al. 2005. *J. Chemother.* **17**, 297; Hail, N. Jr., and Lotan, 2004. *Apoptosis* **9**, 437; Fang, N., and Casida, J.E. 1998. *Proc. Natl. Acad. Sci. USA* **95**, 3380.

3,3′-Dihexyloxacarbocyanine Iodide

[DiOC6(3)]

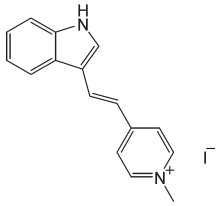
A cationic, cell-permeable, voltage-sensitive, fluorescent carbocyanine dye that is used as a membrane potential probe (excitation max. 484 nm; emission max. 501 nm). Selectively stains mitochondria and the endoplasmic reticulum. Useful as an electron transport inhibitor and as a probe for studying drug accumulation in multi drug-resistant cells.

Cat. No. 305110 **50 mg** **$ 60**

F16

(4-[(E)-2-(Indol-3-yl)ethenyl]-N-methylpyridinium iodide)

A cell-permeable, fluorogenic, delocalized lipophilic cationic compound that acts as a mitochondrial toxin. Possesses the dual ability to induce apoptosis as well as necrosis in tumor cells. Preferentially accumulates in mitochondria, inhibits oxidative phosphorylation and causes mitochondrial transmembrane depolarization. Its incorporation and localization can be monitored by its fluorescence properties. *Excitation max.: ~420 nm, Emission max.: ~520 nm. Purity: ≥ 97% by HPLC.*

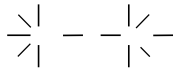


Cat. No. 341246 **25 mg** **$ 138**

Ref.: Fantin, V.R., and Leder, P. 2004. *Cancer Res.* **64**, 329. Fantin, V.R., et al. 2002. *Cancer Cell* **2**, 29.

Ru360

A cell-permeable, oxygen bridged, dinuclear ruthenium amine complex that binds to mitochondria with high affinity (K_d = 340 pM). Specifically blocks Ca^{2+} uptake into mitochondria *in vitro* (IC_{50} = 184 pM).



Cat. No. 557440 **1 mg** **$ 248**

10 x 100 µg **$ 342**

NEW Antibodies for Mitochondrial Research

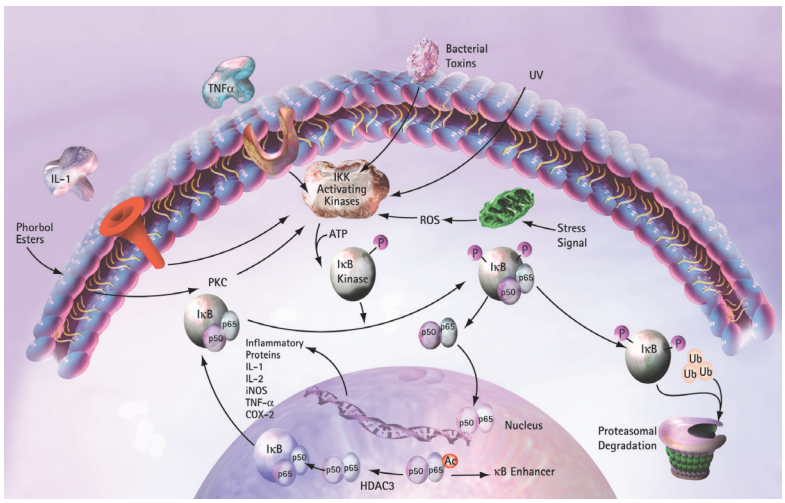
Product	Cat. No.	Comments	Size	Price
Anti-ANT Mouse mAb (5F51BB5AG7)	AP1034	Purified by ammonium sulfate precipitation (1 mg/ml). Recognizes the ~ 30 kDa ANT (adenine nucleotide translocase) protein in cultured fibroblasts. Reacts with bovine, human, and rat. IB (1 µg/ml), IC (5 µg/ml) and IP.	50 µg	220
Anti-Cyclophilin D Mouse mAb (E11AE12BD4)	AP1035	Purified by ammonium sulfate precipitation (1 mg/ml). Recognizes the ~16 kDa cyclophilin D in cultured fibroblasts. Reacts with bovine, human, and rat. IB (1 µg/ml), IC (5 µg/ml)	50 µg	220
Anti-F1F0-a Mouse mAb (7H10BD4F9)	AP1036	Purified by ammonium sulfate precipitation (1 mg/ml). Recognizes the F_1F_0-α subunit of Complex V in bovine heart mitochondria. Reacts with bovine, human, mouse, and rat. IB (1 µg/ml), IC (5 µg/ml), and inhibition studies.	50 µg	220
Anti-F1F0-b Mouse mAb (3D5AB1)	AP1037	Purified by ammonium sulfate precipitation (1 mg/ml). Recognizes the F_1F_0-β subunit of Complex V in bovine heart mitochondria. Reacts with bovine, human, mouse, and rat. IB (0.5 µg/ml), IC (2 µg/ml), IP.	50 µg	220
Anti-GRIM-19 Mouse mAb (6E1BH7)	AP1033	Purified by ammonium sulfate precipitation (1 mg/ml). Recognizes the ~19 kDa GRIM-19 (Genes associated with Retinoid-IFN-induced Mortality) subunit of mitochondrial Complex I (NADH-ubiquinone oxidoreductase) in heart mitochondrial preparations and MRC5 cells. Reacts with bovine, human, mouse, and rat. IB (1 µg/ml), IC (1 µg/ml),	50 µg	220

IB: immunoblotting; **IC:** immunocytochemistry; **IP:** immunoprecipitation; **MAb:** monoclonal antibody; **PAb:** polyclonal antibody

NEW Inhibitors of NF-κB Activation

Nuclear factor-κB (NF-κB)/Rel transcription factors are known to play a pivotal role in inflammatory diseases. Aberrant regulation of NF-κB is also observed in autoimmune disorders and in different types of cancers. Hence, the signaling pathways leading to the regulation of NF-κB activity have become a focal point for drug discovery efforts. NF-κB is normally sequestered in the cytoplasm of non-stimulated cells and consequently must be translocated into the nucleus to function. The subcellular location of NF-κB is controlled by a family of inhibitory proteins known as IκBs, which bind NF-κB and mask its nuclear localization signal thereby preventing its uptake into the nucleus.

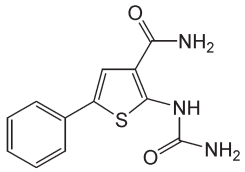
The activation of NF-κB by the extracellular inducers depends on the phosphorylation and subsequent degradation of IκB proteins. Activation of NF-κB is achieved through the action of a family of serine/threonine kinases known as IκB kinase (IKK). The IKK contains two catalytic subunits (IKKα and IKKβ) and a regulatory/adapter protein NEMO (also known as IKKγ). The IKKα and IKKβ phosphorylate IκB proteins and the members of the NF-κB family. All IκB proteins contain two conserved serine residues within their N-terminal area, which are phosphorylated by IKK. IKKα and IKKβ share about 50% sequence homology and can interchangeably phosphorylate Ser^{32}/Ser^{36} of IκBα, and Ser^{19}/Ser^{23} of IκBβ . These phosphorylation events lead to the immediate polyubiquitination of IκB proteins and rapid degradation by the proteasomal pathway. Hence, inhibitors of IKK have long been sought as specific regulators of NF-κB.



IKK-2 Inhibitor VI

(5-Phenyl-2-ureido)thiophene-3-carboxamide)

An ureido-thiophenecarboxamide compound that acts as a potent inhibitor of IKK-2 (IC_{50} = 13 nM). *Purity: ≥98% by HPLC.*

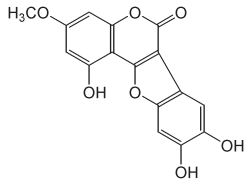


Cat. No. 401483 **1 mg** **$ 117**

Ref.: Baxter, A., et al. 2004. *Bioorg. Med. Chem. Lett.* **14,** 2817.

IKK Inhibitor II, Wedelolactone

A cell permeable, selective, and irreversible inhibitor of IKKα and β kinase activity (IC_{50} < 10 µM). Inhibits NF-κB-mediated gene transcription in cells by blocking the phosphorylation and degradation of IκBα. *Purity: ≥98% by HPLC.*



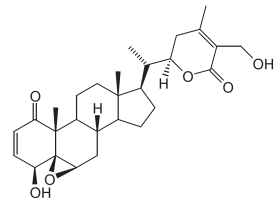
Cat. No. 401474 **1 mg** **$ 77**

Ref.: Kobori, M., et al. 2004. *Cell Death Differ.* **11,** 123; Mors, W.B., et al. 1989. *Toxicon* **27,** 1003.

NF-κB Activation Inhibitor II, JSH-23

(4-Methyl-N¹-(3-phenylpropyl)benzene-1,2-diamine)

A cell-permeable, selective blocker of nuclear translocation of NF-κB p65. Does not affect IκB degradation (IC_{50} = 7.1 µM in LPS-stimulated macrophages RAW 264.7 stably transfected with pNF-κB-SEAP-NPT. *Purity: ≥95% by HPLC.* M.W. 240.3.



Cat. No. 481408 **5 mg** **$ 115**

Ref.: Shin, H.M., et al. 2004. *FEBS Lett.* **571,** 50.

Withaferin A, *Withania somnifera*

A cell-permeable, antitumor, anti-inflammatory, radiosensitizing, and immunosuppressive agent that inhibits angiogenesis (IC_{50} = 12 nM for HUVEC proliferation) and NF-κB activation (IC_{50} = 500 nM in TNF-α-induced endothelial cells) by targeting the ubiquitin-mediated proteasome pathway. *Purity: ≥95% by HPLC.*

Cat. No. 681535 **1 mg** **$ 85**

5 mg **$ 255**

Ref.: Mohan, R., et al. 2004. *Angiogenesis* **7,** 115. Jeyaprakasam, B., et al. 2003. *Life Sci.* **74,** 125. Devi, P.U., et al. 1995. *Cancer Lett.* **95,** 189.

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