

Calbiochem®

# Biologics

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

Merck Biosciences

Calbiochem | Novabiochem | Novagen

Volume 31, No. 1, 2005

## Inside this Issue...

Akt /PI 3-Kinase Signaling in Cell Death and Cell Survival . . . . .	2
NEW! Akt Inhibitors . . . . .	4
NEW! Antibodies for Akt/Protein Kinase B- Related Research. . . . .	5
K-LISA™ Akt Activity Assay Kit . . . . .	6
NEW! Inhibitors of Protein Kinases . . . . .	8
NEW! PhosphoDetect™ Antibodies . . . . .	9
Caveolins: Portal Proteins for Regulated Entry into Cells . . . . .	10
NEW! Angiogenesis Research Tools . . . . .	11
NEW! Cell Migration and Cell Adhesion Assays. . . . .	13
NEW! DNA-Dependent Protein Kinase (DNA-PK) Inhibitors . . . . .	14
K-LISA™ Checkpoint Activity Kit. . . . .	14
Antibodies for DNA Repair and Damage . . . . .	15
NEW! Apoptosis Research Tools . . . . .	16
Neurochemical Corner. . . . .	17
NEW! Protease Assay Kits . . . . .	18
NEW! Antibodies for Proteasome Research . . . . .	19
Highly Purified Enzymes for Ubiquitination . . . . .	20
NEW! Antibodies for Transcription Factors. . . . .	21
InnoZyme™ Myeloperoxidase Activity Kit . . . . .	22
NEW! Technical Tips Section. . . . .	23



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

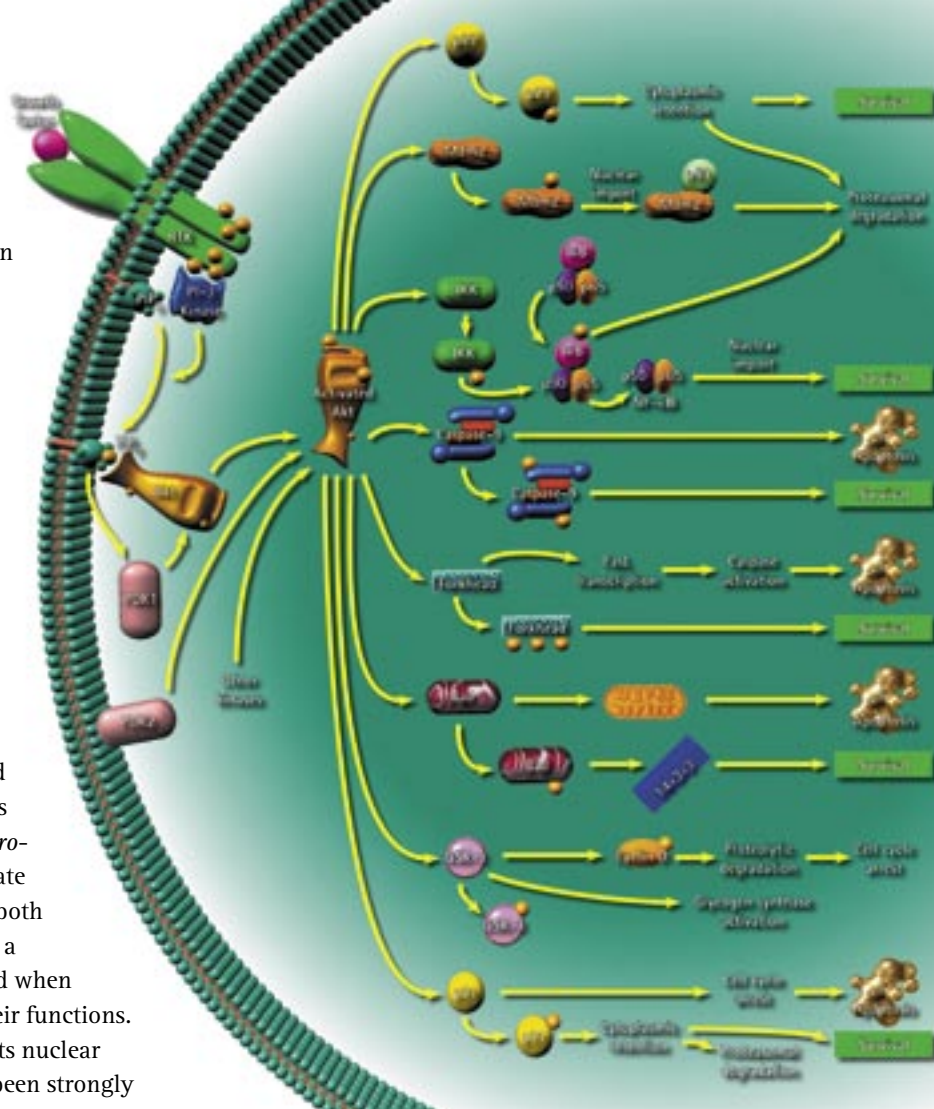
Akt (protein kinase B), a serine/threonine kinase, has emerged as a critical enzyme in signal transduction pathways involved in cell proliferation, apoptosis, angiogenesis, and diabetes. In mammals three isoforms of Akt ( $\alpha$ ,  $\beta$ ,  $\gamma$  or Akt 1, 2, 3) are reported that exhibit a high degree of homology, but differ slightly in the localization of their regulatory phosphorylation sites. Akt $\alpha$  is the predominant isoform in most tissues, whereas the highest expression of Akt $\beta$  is observed in the insulin-responsive tissues, and Akt $\gamma$  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<sub>2</sub> and PIP<sub>3</sub>, a central catalytic domain, and a C-terminal hydrophobic motif.

Akt is constitutively phosphorylated at Ser<sup>124</sup>, in the region between the PH and catalytic domains, and on Thr<sup>450</sup>, in the C-terminal region (in Akt $\alpha$ , 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<sub>2</sub> to generate PIP<sub>3</sub>. The binding of PIP<sub>3</sub> 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<sup>308</sup> and Ser<sup>473</sup> is required for the activation of Akt $\alpha$ , while phosphorylation at Thr<sup>309</sup> and Ser<sup>474</sup> activates Akt $\beta$ . Phosphorylation at Thr<sup>305</sup> activates Akt $\gamma$ . Phosphorylation of a threonine residue on the kinase domain, catalyzed by PDK1, is essential for Akt activation. It causes a charge-induced conformational change, allowing substrate binding and increased rate of catalysis. Akt activity is augmented about 10-fold by phosphorylation at the serine residue by PDK2. DNA-PK and PKC $\beta$  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<sup>136</sup> 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<sup>196</sup>. 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 IKK $\alpha$  at Thr<sup>23</sup> and activate it. The activated IKK $\alpha$ , in turn, phosphorylates I $\kappa$ B, targeting it for ubiquitination and proteasomal degradation. This leads to the activation and nuclear translocation of NF- $\kappa$ B, and transcription of NF- $\kappa$ B-dependent pro-survival genes, including Bcl-x<sub>L</sub> 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<sup>280</sup> it can no longer be phosphorylated by ATM/ATR at Ser<sup>345</sup> 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<sup>353</sup>, resulting in its cytoplasmic accumulation. Cdc25B undergoes activation during S-phase and plays a role in activating the mitotic kinase Cdk1/cyclinB 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. Akt $\alpha$  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<sup>157</sup> 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<sup>145</sup>, causing its localization to the cytoplasm and subsequent degradation.



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

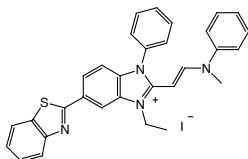
#### References:

- Feng, J., et al. 2004. *J. Biol. Chem.* **279**, 41189.  
 Kawakami, Y., et al. 2004. *J. Biol. Chem.* **279**, 47720.  
 King F.W., et al. 2004. *Cell Cycle* **3**, 634.  
 Franke, T.F., et al. 2003. *Oncogene* **22**, 8983.  
 Zhou, B.-B. S., and Sausville, E.A. 2003. *Prog. Cell Cycle Res.* **5**, 413.  
 Cantley, L.C. 2002. *Science* **296**, 1655.  
 Graff, J.R. 2002. *Expert Opin. Ther. Targets* **6**, 103.  
 Hill, M.M., and Hemmings B.A. 2002. *Pharmacol. Therap.* **93**, 243.  
 Liang, J., et al. 2002. *Nat. Med.* **8**, 1153.  
 Mitsiades, C.S., et al. 2002. *Oncogene* **21**, 5673.  
 Shiojima, I., and Walsh, K. 2002. *Circ. Res.* **90**, 1243.  
 Yang, J., et al. 2002. *Nat. Struct. Biol.* **9**, 940.  
 El-Deiry, W.S. 2001. *Nat. Cell Biol.* **3**, E71.  
 Martin, D., et al. 2001. *J. Neurochem.* **78**, 1000.  
 Mayo, L.D., and Donner, D.B. 2001. *Proc. Natl. Acad. Sci. USA*, **98**, 11598.  
 Somerville, T.C.P., et al. 2001. *Blood* **98**, 1374.  
 Dimmeler, S., and Zeiher, A.M. 2000. *Circ. Res.* **86**, 4.  
 Obata, T., et al. 2000. *J. Biol. Chem.* **275**, 36108.  
 Toker, A., and Newton, A.C. 2000. *J. Biol. Chem.* **275**, 8271.  
 Datta, S.R., et al. 1999. *Genes Dev.* **13**, 2905.  
 Kandel E.S., and Hays, N. 1999. *Exp. Cell Res.* **253**, 210.  
 Peterson, R.T., and Schreiber, S.L. 1999. *Curr. Biol.* **9**, R521.  
 Sabbatini P., and McCormick, F. 1999. *J. Biol. Chem.* **274**, 24263.  
 Alessi, D.R. et al. 1996. *EMBO J.* **15**, 6541.

## Introducing... **NEW!** Akt Inhibitors

### Akt Inhibitor IV

A cell-permeable benzimidazole compound that inhibits Akt phosphorylation/activation by targeting the ATP binding site of a kinase upstream of Akt, but downstream of PI 3-K. Shown to block Akt-mediated FOXO1a nuclear export ( $IC_{50}$  = 625 nM) and cell proliferation ( $IC_{50}$  < 1.25  $\mu$ M) in 786-O cells. Unlike phosphatidylinositol analog-based Akt inhibitors (Cat. Nos. 124005, 124008, 124009), it does not affect PI 3-K activity. *Purity*:  $\geq 98\%$  by HPLC. M.W. 614.6.



Cat. No. 124011	1 mg	€ 110
	5 mg	398

Ref.: Kau, T.R., et al. 2003. *Cancer Cell* 4, 463.

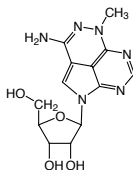
### Akt Inhibitor IV in Solution

A ready-to-use 10 mM (1 mg/163  $\mu$ l) solution of Akt Inhibitor IV (Cat. No. 124011) in DMSO.

Cat. No. 124015	1 mg	€ 112
-----------------	------	-------

### Akt Inhibitor V, Triciribine (NSC 154020)

A cell-permeable tricyclic nucleoside that selectively inhibits the cellular phosphorylation/activation of Akt1/2/3 by targeting an Akt effector molecule other than PI 3-K or PDK. Does not affect PKC, PKA, SGK, Stat3, p38, ERK1/2, or JNK activities. *Purity*:  $\geq 95\%$  by HPLC. M.W. 320.3.



Cat. No. 124012	1 mg	€ 171
-----------------	------	-------

Ref.: Yang, L., et al. 2004. *Cancer Res.* 64, 4394.

Ask us for a free copy of our  
Akt/PI 3-Kinase Signaling  
brochure or visit our website  
[www.calbiochem.com/akt](http://www.calbiochem.com/akt)

### Akt Inhibitor VI, *Akt-in*

#### (H-AVTDHPDRLWAWKEF-OH)

A 15-mer peptide of proto-oncogene TCL1<sub>10-24</sub>, a co-activator of Akt that acts as a specific inhibitor of Akt. Shown to bind to Akt-PH domain ( $K_d$  ~18  $\mu$ M) and interfere with the Akt-phosphoinositide interaction, thus hindering membrane translocation of Akt from the cytosol. *Purity*:  $\geq 95\%$  by HPLC. M.W. 1871.1.

Cat. No. 124013	2 mg	€ 168
-----------------	------	-------

Ref.: Hiromura, M., et al. 2004. *J. Biol. Chem.* 279, 53407.

### Akt Inhibitor VII, TAT-*Akt-in*

#### (H-YGRKKRRQRRRAVTDHPDRLWAWKEF-OH)

A cell-permeable version of the Akt Inhibitor VI, *Akt-in* (Cat. No. 124013) fused with the protein transduction domain TAT that displays anti-tumor properties. Selectively inhibits the phosphorylation of Akt in HEK 293 and QRSP-11 fibrosarcoma cells stimulated with PDGF (complete inhibition at ~50  $\mu$ M). Exhibits minimal inhibitory activity towards PKA, PKC, PDK1, p42/44 MAPK, and p38 MAPK. *Purity*:  $\geq 95\%$  by HPLC. M.W. 3412.9.

Cat. No. 124014	2 mg	€ 306
-----------------	------	-------

Ref.: Hiromura, M., et al. 2004. *J. Biol. Chem.* 279, 53407.

## NOW Available...

### Foxm1b Inhibitor, Cell-permeable (D-Arg)<sub>9</sub>-p19<sup>ARF</sup>26-44)

A cell-permeable p19<sup>ARF</sup>26-44 tumor suppressor peptide that contains an N-terminal membrane transducing nine D-Arginine sequence and inhibits the transcriptional activity of Foxm1b (Forkhead Box m1b). Shown to significantly diminish growth of Foxm1b-transfected U2OS cells, while exhibiting no cytotoxic or apoptotic effects towards non-transfected cells even at 12  $\mu$ M. *Purity*:  $\geq 95\%$  by HPLC. M.W. 3585.3.

Cat. No. 344350	2 mg	€ 300
-----------------	------	-------

Ref.: Kalinichenko, V.V., et al. 2004. *Genes Dev.* 18, 830.



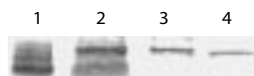
## NEW! Antibodies for Akt/Protein Kinase B-Related Research

Product	Cat. No.	Comments	Size	Price €
Anti-Akt1 (Ab-1), 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. <b>IF</b>	50 µl	269
Anti-Akt1 (88-100), Rabbit pAb	530311	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acids residues 88 - 100 (Cat. No. 530312) of Akt1. Detects a ~60 kDa Akt in a variety of rat and mouse tissues and human cell lines. <b>ELISA, IB, IP</b>	100 µg	341
Anti-Akt, PH Domain, Mouse mAb	ST1088	Monoclonal IgG, protein G-purified. Immunogen used was a GST-fusion protein corresponding to residues 1-149 of human Akt 1. Detects the ~60 kDa Akt in human and rat. <b>FC, IB, IP</b>	50 µg	337
PhosphoDetect™ Anti-Akt1, (pSer <sup>473</sup> ), Mouse mAb	124001	Monoclonal IgG <sub>κ</sub> , immunoaffinity-purified. Clone 11E6. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding the Ser <sup>473</sup> of human Akt1. Recognizes the ~60 kDa Akt1 phosphorylated at Ser <sup>473</sup> in human and mouse. <b>ELISA, IB</b>	10 T	402
PhosphoDetect™ Anti-Akt1, (pThr <sup>308</sup> ), Rabbit pAb	124003	Polyclonal IgG, purified by thiophilic adsorption and size exclusion chromatography. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding the Thr <sup>308</sup> of human Akt1. Recognizes the ~60 kDa human and mouse Akt1 phosphorylated at Thr <sup>308</sup> . Set includes a vanadate treated 224 HepG2 positive control. <b>FC, IB</b>	1 set	376
Anti-Akt2 (Ab-1), 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. <b>IF</b>	50 µl	269
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. <b>ELISA, IB</b>	100 µl	341
Anti-Akt3 (Ab-1), Rabbit pAb	PC512	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 130 - 143 of human and mouse Akt3 protein. <b>IF</b>	50 µl	269
Anti-Akt3, Rabbit pAb	124004	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to a 12-amino acid sequence at the C-terminus of Akt3. Reacts with human, mouse, and rat. <b>ELISA, IB</b>	100 µl	341

**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

### Anti-PDK1, Rabbit pAb

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. Suitable for immunoblotting (1:2000) and immunoprecipitation (5 µl/sample).



Expression of mouse PDK1 isoforms in mouse tissue. Cell lysates (300 µg protein) from mouse testis, liver, SK muscle, and adipocytes (lanes 1, 2, 3, and 4, respectively) were incubated with a goat anti-mouse PDK1 antibody. Immunoprecipitates were separated by SDS-PAGE and the expression of mouse PDK1 isoforms was examined by immunoblotting using Anti-PDK1, Rabbit pAb (Cat. No. ST1036).

**Cat. No. ST1036** **50 µl** **€ 181**

**Ref.:** Dong, L.Q., et al. 1999. *J. Biol. Chem.* **274**, 8117.

### Anti-PI 3-Kinase p110δ, C-Terminal (1026-1044), Rabbit pAb

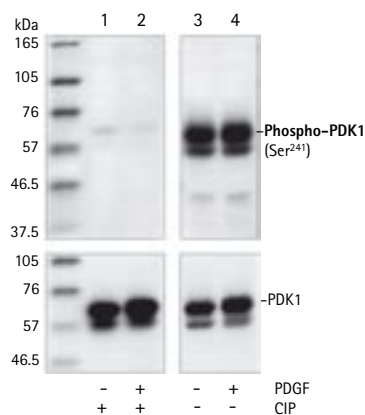
Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide [(C)SWKTKVNWLAHNVSKDNRQ; Cat. No. 526554] corresponding to a distinct C-terminal region of the human phosphatidylinositol 3-kinase p110δ. Suitable for immunoblotting (1:1000) and immunocytochemistry (1:300).

**Cat. No. 526553** **100 µl** **€ 370**

**Ref.:** Vanhaesebroeck, B., et al. 1997. *Proc. Natl. Acad. Sci. USA* **94**, 4330.

### PhosphoDetect™ Anti-PDK1, (pSer<sup>241</sup>), Rabbit pAb

Polyclonal IgG, protein A and peptide affinity purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser<sup>241</sup> of PDK1. Detects the ~63 kDa PDK1 phosphorylated on Ser<sup>241</sup> in human, mouse, and rat. Suitable for immunoblotting (1:1000), immunocytochemistry (1:100), and immunoprecipitation (1:100).



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

**Cat. No. ST1073** **50 µl** **€ 202**

**Ref.:** Williams, M.R., et al. 2000. *Curr. Biol.* **10**, 439. Casamayor, A., et al. 1999. *Biochem. J.* **342**, 287.

### PhosphoDetect™ Anti-PTEN, (pSer<sup>380</sup>), Rabbit pAb

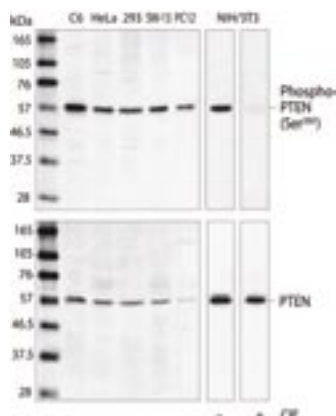
Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser<sup>380</sup> of PTEN. Detects the ~54 kDa PTEN phosphorylated on Ser<sup>380</sup> in human, mouse, and rat. Suitable for immunoblotting (1:1000), immunocytochemistry (1:100), immunoprecipitation (1:50), and immunohistochemistry with paraffin sections (1:50).

Cat. No. ST1072

50 µl

€ 202

Ref.: Sansal, I., and Sellers, W.R. 2004. *J. Clin. Oncol.* **22**, 2954. Birle, D., et al. 2002. *J. Immunol.* **169**, 286. Torres, J., and Pulido, R. 2001. *J. Biol. Chem.* **276**, 993.

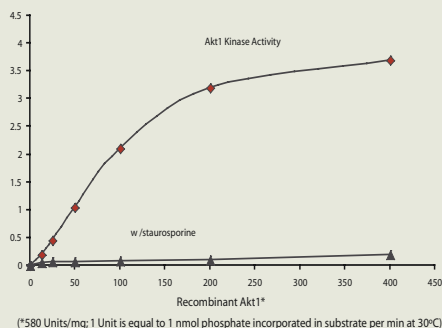


Detection of human PTEN phosphorylated on Ser<sup>380</sup> by immunoblotting. Lysates from C6, HeLa, 293, SW-13, PC-12 (all untreated) and NIH-3T3 cells untreated or treated with calf intestinal alkaline phosphatase (CIP). Primary antibody Phospho-Detect™ Anti-PTEN (pSer<sup>380</sup>), Rabbit pAb (Cat.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

## Looking for Reliable Akt Assay Kits?

### 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 (GRPTSSFAEG) that is phosphorylated on the second serine by Akt1, Akt2, and Akt3.

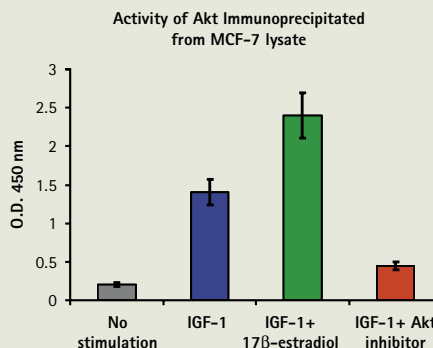


Activity of purified Akt in the presence and absence of staurosporine. The activity of recombinant human Akt1 (Cat. No. 124006) (15 – 400 ng) was determined using the K-LISA™ Akt Activity Kit. Final concentration of staurosporine was 1 µM. Assay range: 10 – 200 ng (580 units/mg).

Cat. No. CBA019

1 kit

€ 447



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 (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 Buffer (Cat. No. 71296-3). Equal amounts of total protein (1.5 mg) were immunoprecipitated and activity was determined.

### Akt Activity Assay Kit

A non-radioactive assay kit for measuring Akt activity in cell lysate or tissue extracts. Akt is first enriched via immunoprecipitation with an anti-Akt antibody and then tested for its ability to phosphorylate GSK-3α, an Akt substrate. Phosphorylated GSK-3α is detected through immunoblotting with anti-GSK-3α phospho-specific antibody.

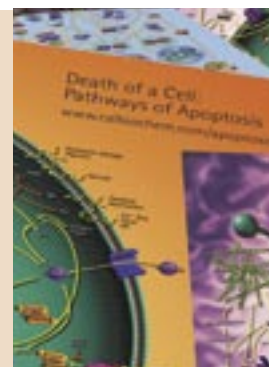
Cat. No. 124007

1 kit

€ 607

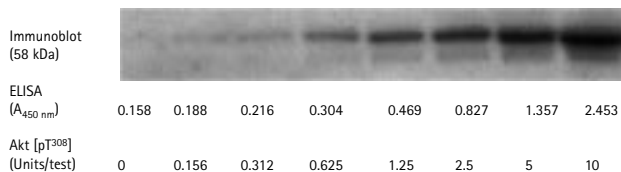
## Have you seen our Interactive Pathways?

Visit us at [www.calbiochem.com/pathways](http://www.calbiochem.com/pathways) and explore our offering of antibodies, assay kits, enzymes, substrates, inhibitors, and much more...



### Akt, Phospho-Specific (Thr<sup>308</sup>) ELISA Kit

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<sup>308</sup>. The sensitivity of this ELISA was compared to Western blotting using known quantities of Akt (pThr<sup>308</sup>). Although this kit was developed for human samples, it has also been found to cross-react with mouse and rat.

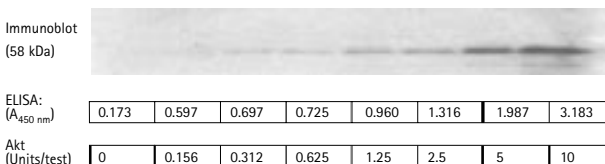


The data presented shows that the sensitivity of this ELISA kit is approximately the same as that of immunoblotting. The bands shown in the immunoblot were developed using PhosphoDetect™ Anti-Akt (pThr<sup>308</sup>), Rabbit pAb (Cat. No. 124001), an alkaline phosphatase conjugated anti-rabbit IgG with a chemiluminescent substrate.

Cat. No. CBA004                      1 kit                      € 718

### Akt, Phospho-Specific (Ser<sup>473</sup>) ELISA Kit

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<sup>473</sup>. Although designed for use with human cell lines, cross-reactivity with mouse and rat cells has also been observed.



The data presented shows that the sensitivity of the ELISA is approximately 2x greater than that of immunoblotting. The bands shown in the immunoblot were developed using PhosphoDetect™ Anti-Akt, (pSer<sup>473</sup>), (Cat. No. 124003), an alkaline phosphatase conjugated anti-rabbit IgG with a chemiluminescent substrate.

Cat. No. CBA005                      1 kit                      € 718

### Akt1 Kinase, His•Tag®, Activated, Human, Recombinant, *S. frugiperda*

A purified recombinant human Akt1 expressed in *Spodoptera frugiperda* cells. Highly active form of Akt1 suitable for labeling Akt substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. *Specific activity: ≥2700 units/mg protein. One unit is defined as the amount of enzyme that will catalyze the transfer of 1.0 pmol of phosphate to the peptide substrate RPRAATF per minute at 30°C. Purity: ≥95% by SDS-PAGE.*

Cat. No. 124006                      20 µg                      € 376

Ref.: Nicholson, K.M., and Anderson, N.G. 2002. *Cell Signal* **14**, 381; Vasquez, F., and Sellers, W.R. 2000. *Biochim. Biophys. Acta* **1470**, M21.

### AKTide-2T (ARKRERTYSFGHHA)

An optimal peptide substrate for assaying Akt/PKB/Rac-protein kinase activity *in vitro*. The peptide undergoes phosphorylation at the Ser site ( $K_m = 3.9 \mu M$ ). Competitively inhibits histone H2B phosphorylation ( $K_i = 12 \mu M$ ) by Akt. *Purity: ≥95% by HPLC.*

Cat. No. 123900                      1 mg                      € 105

Ref.: Obata, T., et al. 2000. *J. Biol. Chem.* **275**, 36108.

### AKTide-SA (ARKRERAYAFGHHA)

Serves as a negative control for AKTide-2T (Cat. No. 123900). Lacks the Ser phosphorylation site. *Purity: ≥95% by HPLC.*

Cat. No. 123905                      1 mg                      € 105

Ref.: Obata, T., et al. 2000. *J. Biol. Chem.* **275**, 36108.

## Involved in Antibody Production?

### Hemocyanin (Blue Carrier), *Concholepa concholepa*

Useful for producing antibodies to haptens and peptides. Exhibits greater water solubility than keyhole limpet hemocyanin.

Cat. No. 374802                      100 mg                      € 149  
(sterile preparation)

Cat. No. 374803                      1 g                      € 356  
(contains 50% glycerol)

Calbiochem®

### PhosphoDetect™ Phospho-specific Antibodies

Antibodies and kits you can rely on for specific detection of phosphorylation events.

Call us or visit our website

[www.calbiochem.com/phosphodetect](http://www.calbiochem.com/phosphodetect)

Order your FREE copy today!

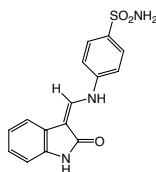






## TrkA Inhibitor

An oxindole compound that acts as a potent and highly selective inhibitor of TrkA ( $IC_{50} = 6 \text{ nM}$ ). Suggested to act by targeting the kinase's ATP binding pocket. Shown to exhibit  $\geq 100$ -fold selectivity over c-fms, Cdk1, Cdk2, Itk, JNK-3, p38, PDHK4, cRaf1, Src, UL13, and VEGFR2. Purity:  $\geq 97\%$  by HPLC. M.W. 315.4.



Cat. No. 648450

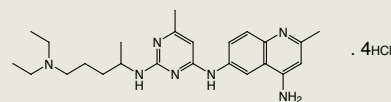
1 mg

€ 116

Ref.: Wood, E.R., et al. 2004. *Bioorg. Med. Chem. Lett.* **14**, 953.

## Rac1 Inhibitor (NSC23766)

A cell-permeable, specific inhibitor of



Rac1 GDP/FTP

exchange activity. Inhibits Rac1-mediated cellular functions in NIH-3T3 and PC-3 cells (effective dose  $\sim 50$  to  $100 \mu\text{M}$ ). Does not affect Cdc42 or RhoA activation or Rac1 interaction with BcrGAP or PAK1.

Purity:  $\geq 95\%$  by HPLC. M.W. 567.4.

Cat. No. 553502

5 mg

€ 224

Ref.: Gao, Y., et al. 2004. *Proc. Natl. Acad. Sci. USA* **101**, 7618.

## NEW! PhosphoDetect™ Antibodies

Although phosphoproteins account for 10-20% of the total proteome, their dynamic nature makes them important regulatory targets in the cell. The ability to determine the state of phosphorylation of specific proteins is of great value in the pursuit to establish the function of a given protein. PhosphoDetect™ (phospho-specific) antibodies are novel tools for qualitative and quantitative detection of phosphorylated proteins without the risks associated with radioactivity. These affinity-purified antibodies are usually depleted for cross-reactivity with non-phosphorylated proteins, which enables them to detect a specific protein in a complex mixture. They recognize the phosphorylated amino acid in the context of the surrounding amino acid sequence. Hence, the degree of phosphorylation of any given protein can be assessed by using a combination of pan antibody and PhosphoDetect™ antibody.

Product	Cat. No.	Comments	Size	Price €
PhosphoDetect™ Anti-Chk1, (pSer <sup>317</sup> ), Rabbit pAb	DR1025	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser <sup>317</sup> of Chk1. Detects the $\sim 56 \text{ kDa}$ Chk1 phosphorylated on Ser <sup>317</sup> in human, monkey, mouse, and rat. <b>IB, IC, PS</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-Chk2, (pThr <sup>68</sup> ), Rabbit pAb	DR1026	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Thr <sup>68</sup> of Chk2. Detects the $\sim 62 \text{ kDa}$ Chk2 phosphorylated on Thr <sup>68</sup> in human and monkey. <b>FC, IB, IC, IP, PS</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-MKK7, (pSer <sup>271</sup> , pThr <sup>275</sup> ), Rabbit pAb	ST1074	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser <sup>271</sup> and Thr <sup>275</sup> of MKK7. Detects the $\sim 48 \text{ kDa}$ MKK7 phosphorylated on Ser <sup>271</sup> and Thr <sup>275</sup> in human. <b>IB</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-Raf, (pSer <sup>259</sup> ), Rabbit pAb	ST1076	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser <sup>259</sup> of c-Raf. Detects the $\sim 74 \text{ kDa}$ c-Raf phosphorylated on Ser <sup>259</sup> in human, mouse, and rat. May detect an additional band at $\sim 68 \text{ kDa}$ . <b>IB, IP, PS</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-SHIP1, (pTyr <sup>1020</sup> ), Rabbit pAb	ST1081	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Tyr <sup>1020</sup> of mouse SHIP1. Detects the $\sim 145 \text{ kDa}$ SHIP1 phosphorylated on Tyr <sup>1020</sup> in mouse or Tyr <sup>1021</sup> in human. <b>IB</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-SHP-2, (pTyr <sup>542</sup> ), Rabbit pAb	ST1082	Polyclonal IgG, immunoaffinity purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Tyr <sup>542</sup> of SHP-2. Detects the $\sim 72 \text{ kDa}$ SHP-2 phosphorylated on Tyr <sup>542</sup> in human, mouse, and rat. <b>IB, IP</b>	50 $\mu\text{l}$	202
PhosphoDetect™ Anti-ATM, (pSer <sup>1981</sup> ), Mouse mAb	DR1002	Monoclonal IgG, protein G-purified. Clone 10H11.E12. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues 1974-1988 of human ATM. Detects the $\sim 370 \text{ kDa}$ ATM protein phosphorylated on Ser <sup>1981</sup> in human and mouse. <b>IB, IC</b>	50 $\mu\text{g}$	181

FC: flow cytometry; IB: immunoblotting; IC: immunocytochemistry; IP: immunoprecipitation; PS: paraffin sections mAb: monoclonal antibody; pAb: polyclonal antibody

## Caveolins: Portal Proteins for Regulated Entry into Cells

Caveolae, flask-shaped invaginations in plasma membrane, serve as membrane organization centers and play important roles in lipid metabolism, growth regulation, signal transduction, tumor suppression, and apoptosis. Three members of the caveolin family (Cav-1, -2, and -3) are essential for the formation of caveolae. These proteins associate with inactive forms of signaling molecules such as Src and Ras family proteins and act as a scaffold for the assembly of signaling complexes. Cav-1 co-localizes and associates with integrin receptors and regulates binding of Src family kinases to the integrin receptors to promote adhesion and anchorage-dependent growth. Cav-2 is abundantly expressed in fibroblasts and differentiated adipocytes, myocytes, and endothelial cells. However, its exact function has not yet been determined. It is co-localized and co-expressed with Cav-1 and requires Cav-1 for proper membrane targeting. It is traditionally considered as a dispensable structural partner of Cav-1. In the absence of Cav-2, caveolae can still form and Cav-1 can maintain its localization in plasma membrane caveolae, but it is partially destabilized. Cav-2-null lung parenchyma shows hypercellularity, with thickened alveolar septa and an increased number of endothelial cells, making Cav-2-null mice exercise intolerant. Cav-3 is expressed exclusively in muscle cells. Monomers of Cav-3 oligomerize to form high molecular mass scaffolding on the cytoplasmic surface of the sarcolemmal membrane. Mutations in Cav-3 are shown to cause limb-girdle muscular dystrophy, rippling muscle disease, distal myopathy, and hyperCKemia.

**References:** Williams, T.M., and Lisanti, M.P. 2004. *Genome Biol.* **5**, 214; Hnasko, R., and Lisanti, M.P. 2003. *Mol. Interv.* **3**, 445; Razani B, et al. 2002. *Mol. Cell Biol.* **22**, 2329; Woodman, S.E., et al. 2004. *Neurology* **62**, 538.

### Anti-Caveolin-2, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acid residues 18 - 31 of human caveolin-2. Detects the ~20 kDa caveolin-2 protein in human. Suitable for immunoblotting (1:500 to 1:2500).

Cat. No. CB1004      50 µg      € 349

### PhosphoDetect™ Antihuman-Caveolin-2, (pSer<sup>23</sup>), Rabbit pAb

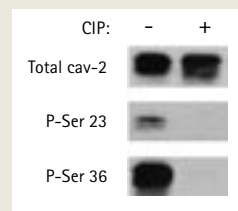
Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues 18 - 31 of human caveolin-2 phosphorylated on Ser<sup>23</sup>. Detects the ~20 kDa caveolin-2 phosphorylated on Ser<sup>23</sup> in human. Suitable for immunoblotting (1:1000 to 1:5000).

Cat. No. CB1005      50 µg      € 365

### PhosphoDetect™ Anti-Caveolin-2, (pSer<sup>36</sup>), Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues 32 - 45 of human caveolin-2 phosphorylated on Ser<sup>36</sup>. Detects the ~20 kDa caveolin-2 phosphorylated on Ser<sup>36</sup> in human. Suitable for immunoblotting (1:500 to 1:5000).

Cat. No. CB1006      50 µg      € 365



Detection of caveolin-2 by immunoblotting. Sample: Lysates (30 µg total protein) from LNCaP cells expressing caveolin-2 incubated without (lane 1) or with (lane 2) calf intestinal alkaline phosphatase (CIP). Primary antibody: Anti-Caveolin-2, Rabbit pAb (Cat. No. CB1004) (top panel), PhosphoDetect™ Anti-Caveolin-2 (pSer<sup>23</sup>), Rabbit pAb (Cat. No. CB1005) (middle panel) and PhosphoDetect™ Anti-Caveolin-2 (pSer<sup>36</sup>) Rabbit pAb (Cat. No. CB1006) (bottom panel). Images provided by Dr. William Sessa of Yale University.

### Anti-Caveolin-2, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acid residues 1-19 of rat and mouse caveolin protein. Recognizes the ~20 kDa caveolin-2 in heart and skeletal muscle of human, mouse and rat. Does not cross-react with caveolin-1 or -3. Suitable for immunoblotting (2 µg/ml) and immunoprecipitation.

Cat. No. 219383      100 µg      € 325

### Caveolin-1 Scaffolding Domain Peptide, Cell-Permeable

A cell-permeable Antennapedia internalization sequence (43-58), fused to caveolin-1 scaffolding domain peptide (C1-SD<sup>82-101</sup>) that is reported to block nitric oxide synthesis, reduce inflammation, matrix invasion, and tumor angiogenesis. It is also shown to enhance endothelial tube formation. The C1-SD<sup>82-101</sup> peptide interacts with several lipid-modified signaling ligands such as EGFR, eNOS, G-protein  $\alpha$ -subunits, PKC $\alpha$ , H-Ras and Src.

Cat. No. 219482 1 mg € 288

Ref.: Bernatchez, P.N., et al. 2005. *Proc. Natl. Acad. Sci. USA* **102**, 761; Williams, T.M., et al. 2004. *J. Biol. Chem.* **279**, 51630; Gratton, J.P., et al. 2003. *Cancer Cell* **4**, 31; Sukumaran, S.K., et al. 2002. *J. Biol. Chem.* **277**, 50716.

### Caveolin-1 Scaffolding Domain Peptide, Cell-permeable, Negative Control

A scrambled caveolin-1 scaffolding domain peptide (C1-SD<sup>82-101</sup>) fused to Antennapedia internalization sequence (43-58) that serves as a useful control for studies employing Caveolin-1 Scaffolding Domain Peptide, Cell-Permeable (Cat. No. 219482).

Cat. No. 219483 1 mg € 205

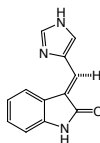
Ref.: Gratton, J.P., et al. 2003. *Cancer Cell* **4**, 31; Sukumaran, S.K., et al. 2002. *J. Biol. Chem.* **277**, 50716.

## NEW! Angiogenesis Research Tools

### Angiogenesis Inhibitor

[(Z,E)-3-(Imidazol-4-ylmethylene)indolin-2-one]

A cell-permeable indolinone compound that displays anti-angiogenic properties (30% inhibition of control at 10  $\mu$ M in an *in vitro* rat aortic ring model) with potency comparable to that of SU5416 (Cat. No. 676487; 22% inhibition of control at 10  $\mu$ M). Acts as a moderate ATP-competitive inhibitor of hEGF-R tyrosine kinase activity (54% inhibition at 10  $\mu$ M). *Purity*:  $\geq 95\%$  by HPLC. M.W. 211.2.

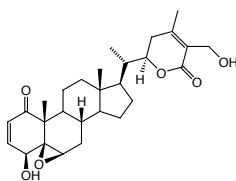


Cat. No. 175580 10 mg € 102

Ref.: Braud, E., et al. 2003. *J. Enzyme Inhib. Med. Chem.* **18**, 243.

### Withaferin A, *Withania somnifera*

A cell-permeable steroidal lactone that acts as a potent inhibitor of angiogenesis (IC<sub>50</sub> = 12 nM in HUVECs proliferation, and 7  $\mu$ g/kg/day in C57BL/6J mice, i.p.) and NF- $\kappa$ B activation (IC<sub>50</sub> = 500 nM in TNF- $\alpha$ -induced endothelial cells) by targeting the ubiquitin-mediated proteasome pathway. *Purity*:  $\geq 98\%$  by HPLC.



Cat. No. 681535 1 mg € 88  
5 mg 300

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

### VEGF Receptor 2 Kinase Inhibitor V, ZM323881

A cell-permeable anilinoquinazoline compound that acts as a potent, reversible, and selective inhibitor of VEGFR-2 (KDR/Flk-1; IC<sub>50</sub> < 2 nM). Has only a trivial effect on VEGFR-1, EGFR, ErbB2, FGFR1, HGFR, and PDGFR $\beta$  even at 50  $\mu$ M levels. Shown to inhibit VEGF-A-induced VEGFR-2 phosphorylation (78% inhibition at 10 nM in frog lung tissue), cell proliferation (IC<sub>50</sub> = 8 nM in HUVEC), and vascular permeability. *Purity*:  $\geq 98\%$  by HPLC. M.W. 375.4.

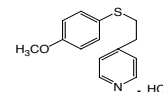
Cat. No. 676497 500  $\mu$ g € 112

Ref.: Endo, A., et al. 2003. *J. Recept. Signal Transduct. Res.* **23**, 239. Whittles, C.E., et al. 2002. *Microcirculation* **9**, 513.

### VEGF Inducer, GS4012

[4-(2-(4-Methoxyphenylsulfanyl)ethyl)pyridine, HCl]

A cell-permeable pyridinyl-thioether that acts as a potent inducer of VEGF and VEGF-mediated vessel formation. GS4012-induced up-regulation of VEGF correlates well with its ability to stimulate tubule network formation (5  $\mu$ g/ml) in HUVECs. *Purity*:  $\geq 97\%$  by HPLC. M.W. 281.8.



Cat. No. 676491 10 mg € 104

Ref.: Peterson, R.T., et al. 2004. *Nat. Biotechnol.* **22**, 595.

## Add convenience to your work...

### Try our ready-to-use Inhibitor Solutions

#### GM6001 in Solution

A 10 mM (1 mg/257 µl) solution of GM6001 (Cat. No. 364205) in DMSO. A potent broad-spectrum hydroxamic acid inhibitor of matrix metalloproteinases (MMPs). Inhibits MMPs *in vitro* ( $K_i$  = 400 pM for MMP-1;  $K_i$  = 500 pM for MMP-2;  $K_i$  = 27 nM for MMP-3;  $K_i$  = 100 pM for MMP-8; and  $K_i$  = 200 pM for MMP-9). *Purity*: ≥95% by HPLC. M.W. 388.5.

Cat. No. 364206      1 mg      € 73

#### VEGF Receptor 2 Kinase Inhibitor III in Solution

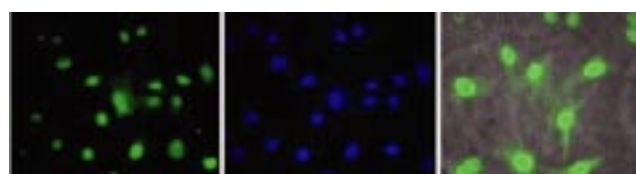
A 10 mM (500 µg/210 µl) solution of VEGF Receptor 2 Kinase Inhibitor III (Cat. No. 676487) in DMSO. Acts as a cell-permeable, selective, ATP-competitive inhibitor of VEGF-R (KDR/Flk-1) and PDGF-R tyrosine kinases ( $IC_{50}$  = 1.04 µM and 20 µM in NIH 3T3 cells overexpressing Flk-1;  $K_m$  = 530 nM for ATP). Inhibition is suggested to be competitive with respect to ATP. *Purity*: ≥95% by HPLC. M.W. 238.3.

Cat. No. 676498      500 µg      € 88

## New! Antibodies for Angiogenesis Research

#### Anti-MTA3, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to a region between amino acid residues 400 and 450 of MTA3. Detects the ~62 kDa MTA3, a protein associated with highly metastatic human carcinomas. Suitable for immunoblotting (1:500 to 1:5000), immunocytochemistry (1:300), and immunoprecipitation (2 to 10 µg/mg lysate).



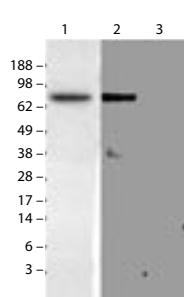
FITC      DAPI      FITC + Phase Contrast  
Detection of human MTA3 by immunocytochemistry. Sample: Sk-Mel-28 cells fixed for 5 min. with ice-cold methanol and blocked with normal goat serum. Primary antibody: Anti-MTA3, Rabbit pAb (Cat. No. IM1012)(1:300). Secondary antibody: Anti-Rabbit IgG, FITC. Detection: fluorescence.

Cat. No. IM1012      50 µg      € 165

Ref.: Fujita, N., et al. 2003. *Cell* **113**, 207; Kumar R. 2003. *Cell* **113**, 142; Simpson, A, et.al. 2001. *Gene* **273**, 29.

#### Anti-Matriptase/MT-SP1, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acid residues near the C-terminus of matriptase. Detects the ~75 kDa matriptase, a type II membrane serine protease, which may play important roles in cell migration and tumor metastasis. Suitable for immunoblotting (1:1000) and immunoprecipitation (10 to 20 µg/mg lysate).



Lane 1: Detection of human Matriptase/MT-SP1 by immunoblotting.  
Sample: Lysates (50 µg) from MCF-7 cells. Primary antibody: Anti-Matriptase/MT-SP1 (Cat. No. IM1014) (1:1000). Secondary antibody: Anti-Rabbit IgG (Goat) Peroxidase Conjugate. Detection: chemiluminescence.

Lanes 2 and 3: Detection of human Matriptase/MT-SP1 by immunoprecipitation (IP) followed by immunoblotting. Sample: Lysate (500 µg) from MCF-7 cells. Antibody for IP (lane 2) Anti-Matriptase/MT-SP1 Rabbit pAb (Cat. No. IM1014) (20 µg/mg total protein). Negative control (lane 3): purified rabbit IgG (20 µg/mg total protein).

Cat. No. IM1014      50 µg      € 165

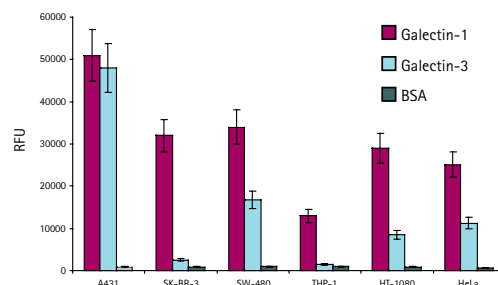
Ref.: Santin, A.D., et al. 2003. *Cancer* **98**, 1898; Takeuchi, T., et al. 2000. *J. Biol. Chem.* **275**, 26333; Lin, C.Y., et al. 1999. *J. Biol. Chem.* **274**, 18231; Takeuchi, T., et al. 1999. *Proc. Natl. Acad. Sci. USA* **96**, 11054.



## NEW! Cell Migration and Cell Adhesion Assays

### InnoCyte™ ECM Cell Adhesion Assay, Galectin-1/Galectin-3

This kit is designed for the determination of the relative attachment of adherent cell lines to galectin-1 and galectin-3, for evaluation of cell adhesion receptors, and for screening cell adhesion antagonists. The kit is supplied with a 96-well strip plate coated with galectin-1 and galectin-3. Cells are seeded in the coated wells and incubated at 37°C. Following incubation, the wells are washed briefly and attached cells are labeled with green fluorescent dye, Calcein-AM (Cat. No. 206700), which is rapidly hydrolyzed by intracellular esterases releasing the membrane impermeant, hydrophilic, intensely fluorescent calcein (*Ex. max:* 485 nm; *Em. max:* 520 nm). BSA-coated wells serve as a negative control and poly-L-lysine-coated wells serve as a positive control for general attachment. Relative cell attachment is assessed using a fluorescence plate reader.



Relative cell attachment of various cell lines to galectin-1, galectin-3, and BSA. Approximately 40,000 cells were added to wells coated with galectin-1, galectin-3, or BSA and incubated for 1.5 h at 37°C in the presence of 6% CO<sub>2</sub>. Cells were washed gently with D-PBS and labeled with Calcein-AM (Cat. No. 206700) for 1 h at 37°C in the presence of 6% CO<sub>2</sub>. HT-1080 cells displayed appreciable binding to poly-L-lysine, which served as a positive control (data not shown). Data presented as relative fluorescence units (RFU).

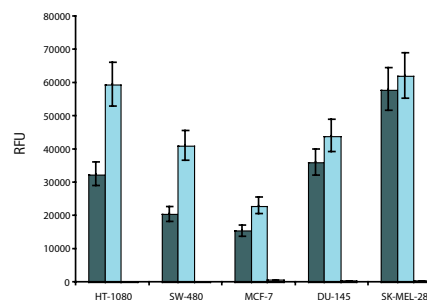
Cat. No. CBA026

1 kit

€ 361

### InnoCyte™ ECM Cell Adhesion Assay, Laminin/Basement Membrane Complex

This kit is designed for the determination of the relative attachment of adherent cell lines to laminin I and basement membrane protein complex, for evaluation of cell adhesion receptors, and for screening cell adhesion inhibitors. The kit is supplied with a 96-well strip plate coated with mouse laminin I and basement membrane complex. Cells are seeded in the coated wells and incubated at 37°C. Following incubation, the wells are washed briefly and attached cells are labeled with the dye, Calcein-AM (Cat. No. 206700). BSA-coated wells serve as a negative control and poly-L-lysine-coated wells serve as a positive control for general attachment. Relative cell attachment is assessed using a fluorescence plate reader.



Relative cell attachment of various cell lines to laminin I, BMC, and BSA. Approximately 40,000 cells were added to wells containing laminin I, BMC, or BSA and incubated for 1.5 h at 37°C in a cell culture incubator in the presence of 6% CO<sub>2</sub>. Cells were washed gently with D-PBS and labeled with Calcein-AM (Cat. No. 206700) for 1 h at 37°C in a cell culture incubator in the presence of 6% CO<sub>2</sub>. Data presented as relative fluorescence units (RFU).

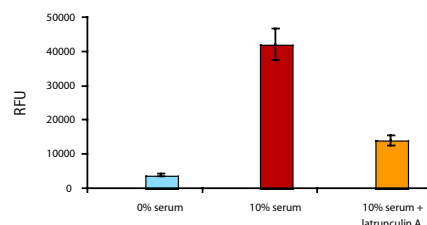
Cat. No. CBA025

1 kit

€ 306

### InnoCyte™ Cell Migration Assay, 24-Well

This kit is suitable for studying the effects of various drugs on cell motility and for identifying chemoattractant agents. The cell culture inserts have an 8 µm pore size membrane that is suitable for migration of epithelial, mesenchymal, and endothelial cell types. Cell migration through the membrane is assessed by staining the cells that attach to the lower side of the membrane with Calcein-AM (Cat. No. 206700), a fluorescent dye, which is rapidly hydrolyzed by intracellular esterases releasing the membrane impermeant, hydrophilic, intensely fluorescent calcein.



Chemotactic migration of HT-1080 cells towards serum in the presence or absence of Latrunculin A (Cat. No. 428021) for 3 h at 37°C. HT-1080 cells were incubated in the presence or absence of serum and Latrunculin A solution for 3 h at 37°C in a 6% CO<sub>2</sub>. Data presented as relative fluorescence units (RFU).

Cat. No. CBA017

1 kit

€ 361

## NEW! DNA-Dependent Protein Kinase (DNA-PK) Inhibitors

DNA-PK is a serine/threonine kinase composed of a large catalytic subunit and two DNA-binding subunits, Ku70 and Ku80. The catalytic subunit is inactive by itself and requires DNA-binding subunits to direct it to DNA and trigger kinase activity. DNA-PK phosphorylates protein targets and also undergoes auto-phosphorylation. The auto-phosphorylation activity has been shown to be essential for repair of random double-strand breaks. DNA-PK phosphorylates p53 on Ser<sup>15</sup> and Ser<sup>37</sup>. Phosphorylation of Ser<sup>15</sup> is suggested to be essential for p53 function. Ser<sup>15</sup> resides within the critical N-terminal region of p53, which

controls the interaction of p53 with the transcriptional apparatus and with the MDM2 protein. Phosphorylation of Ser<sup>15</sup> weakens both the association of p53 with MDM2 and inhibits the repression of p53 by MDM2. Cells defective in DNA-PK components are reported to be hypersensitive to killing by ionizing radiation owing to their inability to repair double-stranded breaks effectively.

### References:

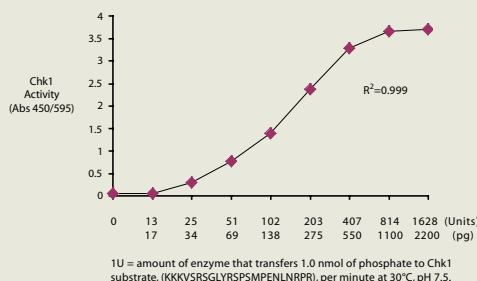
Wechsler, T., et al. 2004. *Proc. Natl. Acad. Sci. USA* **101**, 1247; Basu, A., 2003. *J. Cell. Mol. Med.* **7**, 341; Woo, R.A., et al. 1998. *Nature* **394**, 700; Jackson, S.P., and Jeggo, P.A. 1995. *Trends Biochem. Sci.* **20**, 412.

Product	Cat. No.	Comments	Size	Price €
DNA-PK Inhibitor	260960	A cell-permeable, potent, and selective inhibitor of DNA-PK (IC <sub>50</sub> = 15 µM) and DNA-PK-mediated double-strand breaks.	10 mg	82
DNA-PK Inhibitor II	260961	A cell-permeable, potent, specific, and ATP-competitive inhibitor of DNA-PK (IC <sub>50</sub> = 230 nM). It is highly selective towards DNA-PK over other PI 3-K-related kinases (IC <sub>50</sub> = 13 µM for PI 3-K and >100 µM for ATM and ATR).	5 mg	162
DNA-PK Inhibitor III	260962	A cell-permeable, potent, selective, ATP-competitive inhibitor of DNA-PK (IC <sub>50</sub> = 120 nM) and PI 3-Kinase catalytic subunit p110b (IC <sub>50</sub> = 135 nM). It inhibits DNA-PK-mediated cellular DNA DSB (double-strand break) repair (EC <sub>50</sub> = 68 µM).	1 mg	100
DNA-PK Inhibitor IV	260963	A potent, selective, and ATP-competitive inhibitor of DNA-PK (IC <sub>50</sub> = 430 nM). Inhibits PI 3-Kinase catalytic subunit p110-isozymes at higher concentrations (IC <sub>50</sub> = 10 µM, 2.8 µM, 5.1 µM and 37 µM for α, β, δ and γ, respectively).	1 mg 5 mg	88 281
DNA-PK Inhibitor V	260964	A potent, selective, and ATP-competitive inhibitor of DNA-PK (IC <sub>50</sub> = 270 nM). Inhibits PI 3-Kinase catalytic subunit p110-isozymes at higher concentrations (IC <sub>50</sub> = 32 µM, 3.7 µM, 22 µM and ~ 100 µM for α, β, δ and γ, respectively).	1 mg 5 mg	106 350

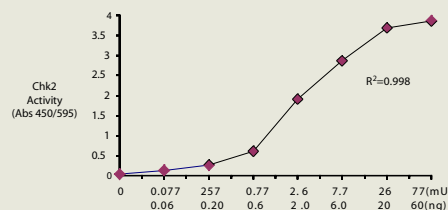
## Studying Cell Cycling? Check out our New...

### K-LISA™ Checkpoint Activity Kit

A rapid, sensitive, 96-well ELISA-based activity 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 (KKKVSRSGLYRSPSPMPENLN RPR) that is phosphorylated on the third serine by Chk1 and Chk2. The phosphorylated substrate is detected with a phosphoserine detection antibody, followed by anti-IgG HRP conjugate and color development with TMB substrate. Addition of inhibitor (Staurosporine; Cat. No. 569397) serves as a negative control.



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).

Cat. No. CBA020

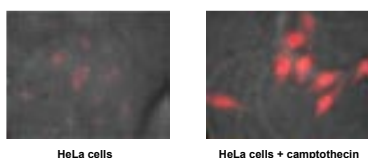
1 kit

€ 447

## Interested in Antibodies for DNA Repair and Damage?

### PhosphoDetect™ Anti-ATM, (pSer<sup>1981</sup>), Mouse mAb (10H11•E12)

Monoclonal IgG<sub>1</sub>, purified. Clone 10H11.E12. Immuno-gen used was a synthetic phosphopeptide corresponding to amino acid residues 1974 to 1988 (phosphorylated on Ser<sup>1981</sup>) of human ATM. Detects the ~370 kDa ATM protein when phosphorylated on Ser<sup>1981</sup>. Activation of ATM kinase by auto-phosphorylation at Ser<sup>1981</sup> has been reported to be an initiating event in the cellular response to radiation. Reacts with human and mouse. Suitable for immunoblotting (0.5 µg/µl) and immunocytochemistry (1.5 µg/µl).



Detection of human phosphorylated ATM (Ser<sup>1981</sup>) by immunofluorescence. Samples: Untreated HeLa cells (left panel) and camptothecin-treated (10 µM) HeLa cells using PhosphoDetect™ Anti-ATM, pSer<sup>1981</sup>, Mouse mAb (Cat. No. DR1002). Secondary antibody used was Goat anti-mouse IgG, AlexaFluor 546.

Cat. No. DR1002 50 µg € 181

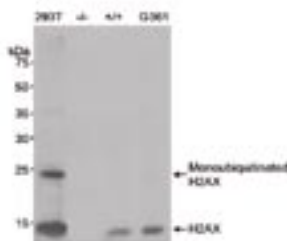
### Anti-Pso4, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to a region of Pso4 near the N-terminus. Detects the ~55 kDa Pso4, a ubiquitously expressed protein that plays a major role in DNA repair. May also immunoprecipitate an additional band at ~110 kDa. Useful for immunoblotting (1:1000) and immunoprecipitation (1:100). Supplied at 1 mg/ml.

Cat. No. DR1022 50 µg € 165

### Anti-H2AX, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to a portion of the C-terminus of H2AX (LocusLink ID 3014). Detects the ~15 kDa H2AX and its ~24 kDa mono-ubiquitinated form in human and mouse. Phosphorylation of H2AX on Ser<sup>139</sup> is an early event in the response to DNA damage. Suitable for immunoblotting (1:1000) and immunoprecipitation (5 to 20 µg/mg lysate).

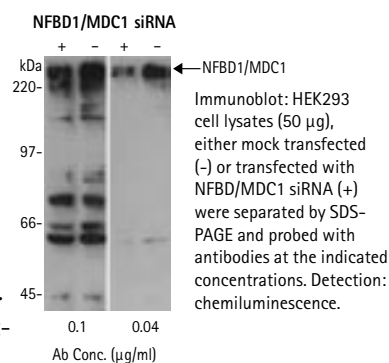


Total protein from nuclear extracts from human 293T cells, H2AX knockout (-/-), H2AX wild-type (+/+), and human melanoma G-361 cells were separated via SDS-PAGE and probed with Anti-H2AX, Rabbit pAb (Cat. No. DR1016) at 1:1000 dilution. Detection: chemiluminescence.

Cat. No. DR1016 100 µg € 324

### Anti-NFBD1/MDC1, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide representing a portion of human NFBD1/MDC1 encoded within exon 10. Detects the ~250 kDa NFBD1/MDC1 in human. Suitable for immunoblotting (1:5000 to 25,000) and immunoprecipitation (2 to 4 µg/mg lysate).

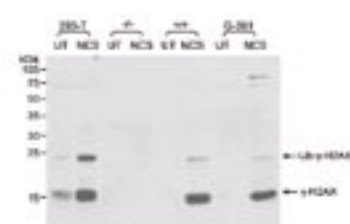


Immunoblot: HEK293 cell lysates (50 µg), either mock transfected (-) or transfected with NFBD1/MDC1 siRNA (+) were separated by SDS-PAGE and probed with antibodies at the indicated concentrations. Detection: chemiluminescence.

Cat. No. DR1018 100 µg € 324

### PhosphoDetect™ Anti-H2AX, (pSer<sup>139</sup>), Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide surrounding the phosphorylated Ser<sup>139</sup> of H2AX. Detects the ~15 kDa H2AX and the ~24 kDa monoubiquitinated form phosphorylated on Ser<sup>139</sup> in human and mouse. Phosphorylation of H2AX on Ser<sup>139</sup>



Total protein from nuclear extracts of untreated (UT) or samples treated with neocarcinostatin (NCS) at 200 ng/ml for 30 min., human 293T cells, H2AX knockout (-/-), H2AX wild-type (+/+), and human melanoma G-361 cells were separated and probed with PhosphoDetect™ Anti-H2AX, (pSer<sup>139</sup>), Rabbit pAb (Cat. No. DR1017) at 1:10,000 dilution. Detection: chemiluminescence.

is an early event in the response to DNA damage. Suitable for immunoblotting (1:5000 to 1:50,000) and immunocytochemistry (1:400 to 800). Supplied at 1 mg/ml.

Cat. No. DR1017 100 µg € 361

## NEW! Caspase Inhibitor

### Caspase-3/7 Inhibitor II

A potent, reversible and active site binding inhibitor of caspases-3 and -7 (IC<sub>50</sub> = 3.2 nM and 22.6 nM, respectively) and displays ~100-fold greater selectivity over caspases-8 and -9 (IC<sub>50</sub> = 577.6 nM and 364.7 nM, respectively). Purity: Single main spot with additional trace spot by TLC.

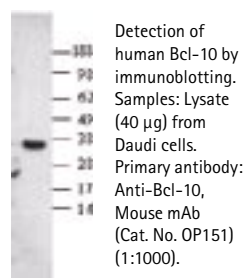
Cat. No. 218832 1 mg € 104

## NEW! APOPTOSIS Research Tools

### Anti-Bcl-10, Mouse mAb

Monoclonal IgG, Clone 151. Supplied as undiluted ascites. Immunogen used was full length recombinant Bcl-10.

Epitope lies in the region of amino acids 168 - 233 of human Bcl-10. Detects the ~32 kDa Bcl-10 in human. May detect an additional band at ~37 kDa. Suitable for immunoblotting (1:1000), and paraffin sections (1:60).



Cat. No. OP151

50 µl

€ 165

### PhosphoDetect™ Anti-MDM2, (pSer<sup>166</sup>), Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser<sup>166</sup> of MDM2. Detects the ~90 kDa MDM2 when phosphorylated on Ser<sup>166</sup> in human, mouse, and rat.

Phosphorylation of MDM2 blocks its binding to p19<sup>ARF</sup> increasing the degradation of p53. Suitable for immunoblotting (1:1000).



Detection of human MDM2 phosphorylated on Ser<sup>166</sup> by immunoblotting. Samples: Lysates from MCF-7 cells treated with IGF-1 for the indicated time periods. Primary antibody: PhosphoDetect™ Anti-MDM2, (pSer<sup>166</sup>), Rabbit pAb (Cat. No. DR1027).

Cat. No. DR1027

50 µl

€ 202

### Presenilin 1, CT-15 Peptide, Cell-permeable (RQIKI-WFQNRRMKWKK-VQPFMQDLAFHQFYI)

A cell-permeable presenilin 1 (PS1) C-terminus 15-residue peptide fused to the protein transduction domain of Antennapedia homeodomain. Acts as an activator of Omi/HtrA2 protease activity, binds to the PDZ domain of Omi/HtrA2, and increases its proteolytic activity towards inhibitor of apoptosis proteins (IAPs) and β-casein. Shown to induce cell death in an Omi/HtrA2-dependent manner in 293T, MCF-7 and mnd2-Omi/HtrA2 cells.

Purity: ≥95% by HPLC. M.W. 4112.9.

Cat. No. 529589

1 mg

€ 229

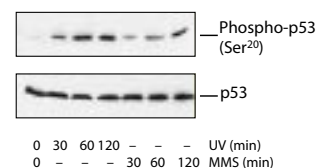
Ref.: Gupta, S., et al. 2004. *J. Biol. Chem.* **279**, 45844.

### PhosphoDetect™ Anti-p53, (pSer<sup>20</sup>), Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser<sup>20</sup> of p53.

Detects p53 only when phosphorylated on Ser<sup>20</sup> in human and mouse.

Suitable for immunoblotting (1:1000), immunocytochemistry (1:200; fluorescence), and paraffin sections (1:50).



Detection of monkey p53 phosphorylated on Ser<sup>20</sup> by immunoblotting. Samples: Lysates from COS cells treated with UV light or the DNA damaging agent, methyl methanesulfone (MMS). Primary antibodies: PhosphoDetect™ Anti-p53 (pSer<sup>20</sup>) Rabbit pAb (Cat. No. DR1023) (1:1000) (upper panel) and Anti-p53, total (lower panel).

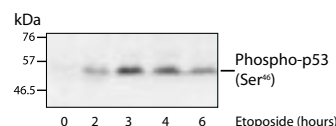
Cat. No. DR1023

50 µl

€ 202

### PhosphoDetect™ Anti-p53, (pSer<sup>46</sup>), Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Ser<sup>46</sup> of p53. Detects p53 when phosphorylated on Ser<sup>46</sup> in human. Suitable for immunoblotting (1:1000), immunocytochemistry (1:1000), and immunoprecipitation (1:500).



Detection of human p53 phosphorylated on Ser<sup>46</sup> by immunoblotting. Sample: Cell lysates from MCF-7 cells treated with Etoposide (Cat. No. 341205). Primary antibody: PhosphoDetect™ Anti-p53, Phospho-Specific (pSer<sup>46</sup>), Rabbit pAb (Cat. No. DR1024) (1:1000).

Cat. No. DR1024

50 µl

€ 202

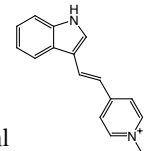
### F16

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

A cell-permeable, fluorogenic, mitochondrial toxin that possesses the ability to induce apoptosis as well as necrosis in tumor cells. Preferentially accumulates in mitochondria, inhibits oxidative phosphorylation and causes mitochondrial transmembrane depolarization.

Ex. max.: ~420 nm, Em. max.: ~520 nm.

Purity: ≥97% by HPLC. M.W. 362.2.



Cat. No. 341246

25 mg

€ 168

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



## Neurochemical Corner

### $\alpha$ -APP Modulator

**(2S,5S)-(E,E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadienylamino)benzolactam**

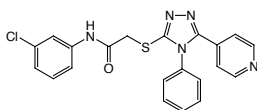
A cell-permeable benzolactam derivative that enhances non-amyloidogenic  $\alpha$ -processing of amyloid precursor protein (APP) (at 100 nM in human fibroblast AG06848). Also acts as an activator of PKC ( $K_i = 11.9$  nM for PKC $\alpha$ ). *Purity:  $\geq 95\%$  by HPLC. M.W. 501.5.*

Cat. No. 565740                      1 mg                      € 179

Ref.: Kozikowski, A.P., et al. 2003. *J. Med. Chem.* **46**, 364.

### Neurokinin-1 Receptor Antagonist (ASN-1377642)

A triazolyl-amide compound that acts as a high-affinity ( $K_i = 251$  nM) antagonist for NK1, the Substance P (Cat. No. 05-23-0600)-specific neurokinin receptor. Shown to compete with Substance P binding to NK1 on whole CHO cells. *Purity:  $\geq 95\%$  by HPLC. M.W. 421.9.*

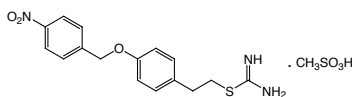


Cat. No. 480736                      1 mg                      € 138

Ref.: Evers, A., and Klebe, G. 2004. *Angew. Chem. Int. Ed.* **43**, 248.

### KB-R7943

A cell-permeable inhibitor of the influx/reverse mode of Na<sup>+</sup>/Ca<sup>2+</sup> exchange (NCX; IC<sub>50</sub> = 4.3  $\mu$ M, 4.7  $\mu$ M, and 1.4  $\mu$ M for NCX1, NCX2, and NCX3, respectively) that directly modulates Na<sup>+</sup>/Mg<sup>2+</sup> exchange in a Ca<sup>2+</sup>-dependent manner. Reported to offer neuronal and cardioprotection. Also inhibits nicotinic acetylcholine receptors and NMDA receptor channels (IC<sub>50</sub> < 10  $\mu$ M). *Purity:  $\geq 98\%$  by HPLC. M.W. 427.5.*



Cat. No. 420336                      5 mg                      € 118

Ref.: Hobai, I.A., and O'Rourke, B. 2004. *Expert Opin. Investig. Drugs* **13**, 653; Uetani, T., et al. 2003. *J. Biol. Chem.* **278**, 47491; Iwamoto, T., et al. 2001. *Mol. Pharmacol.* **59**, 524; Pintado, A.J., et al. 2000. *Br. J. Pharmacol.* **130**, 1893.

### $\beta$ -Secretase Substrate VIII, Fluorogenic (Abz-VNL~DAE-EDDnp)

An internally quenched fluorogenic peptide substrate designed from Swedish-mutated  $\beta$ -amyloid precursor protein sequence that specifically detects the activity of BACE1 ( $\beta$ -secretase 1) and BACE2 ( $\beta$ -secretase 2). Cleavage occurs between Leu-Asp residues, which results in enhancement of fluorescence. Useful for screening of inhibitors for BACE1 and BACE2. *Purity:  $\geq 98\%$  by HPLC. M.W. 987.0.*

Cat. No. 565783                      1 mg                      € 114

Ref.: Andrau, D., et al. 2003. *J. Biol. Chem.* **278**, 25859.

### $\gamma$ -Secretase Inhibitor IX in Solution

A 25 mM (5 mg/462  $\mu$ l) solution of  $\gamma$ -Secretase Inhibitor IX (Cat. No. 565770) in DMSO. *Purity:  $\geq 95\%$  by HPLC.*

Cat. No. 565784                      5 mg                      € 110

## NEW! Antibodies for Neurochemical Research

### Anti- $\beta$ -Amyloid, Rabbit pAb

Polyclonal IgG, purified. Immunogen used was a synthetic peptide corresponding to amino acid residues 3 - 16 of mouse  $\beta$ -amyloid. Reacts with all isoforms of rat and mouse  $\beta$ -amyloid. Exhibits negligible cross-reactivity with human  $\beta$ -amyloids. Suitable for immunoblotting (1:1000) and immunohistochemistry (1:200 to 1:1000 on frozen sections). Supplied at 1 mg/ml.

Cat. No. NE1012                      50  $\mu$ l                      € 177

### PhoshoDetect™ Anti-Tyrosine Hydroxylase, (pSer<sup>31</sup>), Rabbit pAb

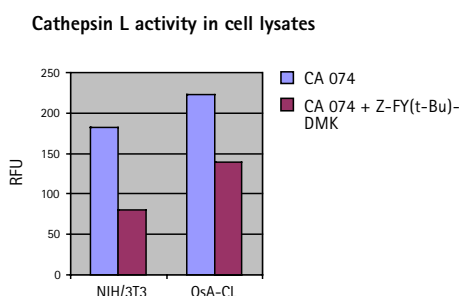
Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to amino acid residues surrounding phosphorylated Ser<sup>31</sup> of tyrosine hydroxylase. Detects the ~60 kDa tyrosine hydroxylase phosphorylated on Ser<sup>31</sup> in rat, does not detect the unphosphorylated protein. Phosphorylation of tyrosine hydroxylase on Ser<sup>31</sup> in certain brain regions is shown to increase by electrical stimulation, extracellular signal-regulated protein kinase activity, and with haloperidol or clozapine treatment. Suitable for immunoblotting (1:1000).

Cat. No. NE1001                      100  $\mu$ l                      € 409

## Introducing...**NEW!** Protease Assay Kits

### InnoZyme™ Cathepsin L Activity Kit, Fluorogenic

A highly sensitive and selective fluorogenic assay for cathepsin L activity in cell lysates, tissue extracts, and purified enzyme preparations. The kit includes Z-Phe-Arg-AMC as a fluorogenic substrate (*Ex. max.*: = 360 nm; *Em. max.*: = 460 nm) and a cathepsin L inhibitor, Z-Phe-Tyr(t-Bu)-DMK. Interference from cathepsin B is eliminated by the incorporation of CA-074 (Cat. No. 205530), a specific, irreversible inhibitor of cathepsin B. The 96-well format provides a convenient platform for screening cathepsin L inhibitors. Interference from other lysosomal cysteine proteinases is minimal: < 1 % for human cathepsin B, H, and S, and < 2 % for human Cathepsin K. Detection range: 1.56 to 100 ng/ml.

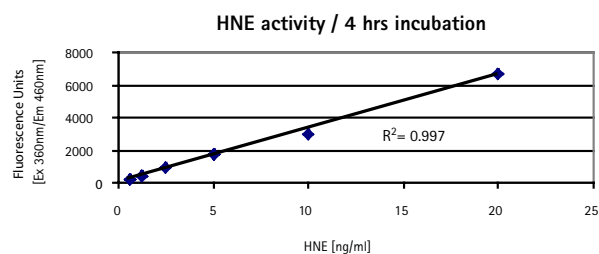


Cathepsin L activity in cell lysates reported as relative fluorescence units (RFU). Sample: NIH-3T3, OsA-CL (human osteosarcoma) cells. Cells were grown in MEM or RPMI 1600 medium supplemented with 10% fetal calf serum and harvested at 70 to 90% confluency. Cell lysates were prepared using CytoBuster™ Protein Extraction Reagent (Cat. No. 71009). Total protein was determined using a BCA protein assay. Cathepsin L activity displayed was calculated by subtracting the RFU value derived in the presence of Cathepsin B inhibitor, CA-074 + Cathepsin L inhibitor (Z-FY (t-Bu)-DMK) from the RFU value derived only in the presence of CA-074.

Cat. No. CBA023      1 kit      € 447

### InnoZyme™ Human Neutrophil Elastase Immunocapture Activity Assay Kit

A sensitive and selective assay kit for human neutrophil elastase (HNE). The kit utilizes anti-HNE immobilized onto a 96-well plate; activity is measured with fluorogenic substrate, MeOSuc-Ala-Ala-Pro-Val-AMC. Cleaved AMC is measured fluorometrically (*Ex. max.* 360-380 nm; *Em. max.*: 440-460 nm). This kit is suitable for use with cell lysates and body fluids and for screening HNE inhibitors. Detection range: 0.625 to 20 ng/ml.



Cat. No. CBA016      1 kit      € 483

## Now Available...

### Renin, Human, Recombinant

Secreted by the juxtaglomerular cells that acts 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 function. *Purity: ≥99% by SDS PAGE. M.W. 40,000.*

Cat. No. 553900      5 µg      € 116  
10 µg      190

## NEW! Protease Inhibitors

Product	Cat. No.	Comments	Size	Price €
Aminopeptidase N Inhibitor (2',3-Dinitroflavone-8-acetic acid)	164602	A selective, reversible, and competitive inhibitor of aminopeptidase N (APN/CD13; IC <sub>50</sub> = 25 µM in U937 cells). Displays ~ 2 - 3 fold lower inhibition potency than Bestatin (Cat. No. 200484), but is not cytotoxic. <i>Purity: ≥98% by HPLC.</i>	5 mg	110
Coronavirus Main Proteinase Inhibitor (CBz-VNSTLQ-CMK)	235035	An irreversible substrate-analog inhibitor of several viral proteinases. Shown to covalently modify the active site cysteine residue. The peptide is derived from the P6 - P1 residues of the NH <sub>2</sub> -terminal autoprocessing site of porcine TGEV M <sup>pro</sup> (transmissible gastroenteritis virus main proteinase) and is expected to bind to all other coronavirus homologs, such as human SARS-CoV M <sup>pro</sup> and HCoV 229E M <sup>pro</sup> , in a similar manner and with similar affinity. <i>Purity: ≥95% by HPLC.</i>	1 mg 5 mg	120 448
Elastase Inhibitor IV (N-(o-(p-Pivaloyloxybenzene) sulfonylamino benzoyl)glycine)	324759	A cell-permeable, potent, substrate-competitive, and highly specific inhibitor of neutrophil elastase (IC <sub>50</sub> = 19 - 49 nM). Displays >100-fold greater selectivity over pancreatic elastase (IC <sub>50</sub> = 5.6 µM). <i>Purity: ≥95% by HPLC.</i>	1 mg	80

### Protease Inhibitor Cocktail Set III

This EDTA-free cocktail is recommended for use with mammalian cells and tissue extracts. Each vial contains 100 mM AEBSEF, HCl (Cat. No. 101500), 80  $\mu$ M Aprotinin, Bovine Lung (Cat. No. 616398), 5 mM Bestatin (Cat. No. 200484), 1.5 mM E-64 Protease Inhibitor (Cat. No. 324890), 2 mM Leupeptin, Hemisulfate (Cat. No. 108975), and 1 mM Pepstatin A (Cat. No. 516482). Provided in 1 ml of DMSO.

Cat. No. 539134	1 ml	€ 68
	1 set (5 x 1 ml)	294

### Protease Inhibitor Cocktail Set VIII

A cocktail of three protease inhibitors provided in DMSO. This cocktail is designed to inhibit cysteine proteases, including calpains, cathepsins, and papain. Each vial contains 1.56 mM ALLN (Cat. No. 208719), 1.5 mM E-64 Protease Inhibitor (Cat. No. 324890), and 0.5 mM Cathepsin Inhibitor I (Cat. No. 219415).

Cat. No. 539129	1 ml	€ 74
	1 set (5 x 1 ml)	318

## Complex Nature of the Proteasome Complex

The proteasome is a 26S complex that contains a 20S proteasome core, a multi-catalytic protease complex, and a 19S complex containing several ATPases and a binding site for ubiquitin chains. The proteolytic core of this complex, the 20S proteasome, contains multiple peptidase activities and functions as the catalytic machine. This core is composed of 28 subunits arranged in four heptameric, tightly stacked rings ( $\alpha$ 7,  $\beta$ 7,  $\beta$ 7,  $\alpha$ 7) to form a cylindrical structure. The  $\alpha$ -subunits (25.8 kDa) make up the two outer, and the  $\beta$ -subunits (22.3 kDa) the two inner rings of the stack. The entrance of substrate proteins to the active site of the complex is guarded by the  $\alpha$ -subunits that only allow access for unfolded and extended polypeptides. The proteolytic activity is confined to the  $\beta$ -subunits. Binding studies have shown 14 catalytic sites within the central chamber and involve a novel proteolytic mechanism in which the hydroxyl group of a threonine, located at the N-terminus of the  $\beta$ -subunit, acts as the nucleophilic group in the peptide hydrolysis. The proteolytic activity of the proteasome appears to be rather unspecific, however, the size of the hydrolysis products is always between 6 to 9 residues, which corresponds to the length between adjacent catalytic sites in the central chamber.

## NEW! Antibodies for Proteasome Research

Product	Cat. No.	Comments	Size	Price €
Anti-Hip-2, Rabbit pAb	NE1011	Polyclonal IgG, undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acid residues 1-12 of E2-25K/Hip-2. Detects the ~25 kDa E2-25K/Hip-2, an ubiquitin conjugating enzyme that plays a role in mediating amyloid- $\beta$ neurotoxicity. Reacts with human, mouse, and rat. <b>FS, IB, IP, PS</b>	50 $\mu$ l	165
Anti-20S Proteasome $\alpha$ 7-Subunit, Mouse mAb	ST1052	Monoclonal IgG <sub>1</sub> , partially purified. Clone MCP72. S. Immunogen used was dinitrophenylated human placenta derived proteasomes. Detects ~ the 30 kDa 20S proteasome $\alpha$ 7-subunit protein in human, rat, rabbit, and yeast. <b>IB, PS</b>	100 $\mu$ l	349
Anti-20S Proteasome Core Subunits, Rabbit pAb	ST1053	Polyclonal IgG, undiluted serum. Immunogen used was human erythrocyte-derived proteasomes. Detects ~25-30 kDa 20S proteasome core subunits ( $\alpha$ 5, $\alpha$ 7, $\beta$ 1, $\beta$ 5i, and $\beta$ 7) in human, mouse, and yeast. <b>IB, IP, PS</b>	100 $\mu$ l	349
Anti-20S Proteasome $\beta$ 1-Subunit, Mouse mAb	ST1054	Monoclonal IgG <sub>1</sub> , partially purified. Clone MCP421. Immunogen used was dinitrophenylated human placenta-derived proteasomes. Detects the ~29 kDa 20S proteasome $\beta$ 1-subunit protein in human and rabbit. <b>IB</b>	100 $\mu$ l	349
Anti-20S Proteasome $\beta$ 3-Subunit, Mouse mAb	ST1055	Monoclonal IgG <sub>1</sub> , partially purified. Clone MCP102. Immunogen used was dinitrophenylated proteasomes. Detects the ~23 kDa 20S proteasome $\beta$ 3-subunit protein in human, mouse, rabbit, and rat. <b>IB</b>	100 $\mu$ l	349
Anti-20S Proteasome $\beta$ 4-Subunit, Rabbit pAb	ST1056	Polyclonal IgG, partially purified. Immunogen used was a synthetic peptide corresponding to amino acids residues 72 - 85 of human proteasome subunit $\beta$ 4 (Accession No. P49721) conjugated to KLH. Detects the ~23 kDa 20S Proteasome $\beta$ 4-Subunit protein in human, mouse, and rat. <b>IB</b>	100 $\mu$ l	349
Anti-20S Proteasome $\beta$ 5i-Subunit, Rabbit pAb	ST1057	Polyclonal IgG, partially purified. Immunogen used was recombinant protein corresponding to amino acids residues 23 - 223 of murine proteasome subunit $\beta$ 5i. Detects the ~26 kDa 20S proteasome subunit $\beta$ 5i, a subunit of the immunoproteasome in human, mouse, and rabbit. <b>IB, PS</b>	100 $\mu$ l	349
Anti-STAM1, Rabbit pAb	ST1040	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to the C-terminal region of STAM1. Detects the ~68 kDa STAM1 in human and mouse. STAM1 is a cytoplasmic adaptor protein that plays a major role in the sorting of ubiquitinated proteins. <b>IB, IP</b>	50 $\mu$ g	181
Anti-STAM2, Rabbit pAb	ST1038	Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to the C-terminal region of STAM2. Detects the ~58 kDa STAM2 in human and mouse. STAM2 plays a major role in the sorting of ubiquitinated proteins. <b>IB, IP</b>	50 $\mu$ g	168

**FS:** frozen sections; **IB:** immunoblotting; **IP:** immunoprecipitation; **PS:** paraffin sections; **mAb:** monoclonal antibody; **pAb:** polyclonal antibody

## Looking for Highly Purified Enzymes for Ubiquitination?

### Ubiquitin-Activating Enzyme E1, Human

The ubiquitin-activating enzyme, E1, is required for initiating a multi-step pathway for the covalent linkage of ubiquitin to target proteins. It catalyzes the first step in the ubiquitin-protein isopeptide bond formation and is a critical component for the initiation of conjugation reactions *in vitro*. Purity:  $\geq 98\%$  by SDS-PAGE.

Cat. No. 662072                      25  $\mu$ g                      € 245

### Ubiquitin-Activating Enzyme E1, GST-Fusion Protein, Human

Cat. No. 662071                      50  $\mu$ g                      € 245

Ref.: Ciechanover, A., et al. 1982. *J. Biol. Chem.* **257**, 2537; Haas, A.L., et al. 1982. *J. Biol. Chem.* **257**, 10329; Ciechanover, A., 1981. *Proc. Natl. Acad. Sci.* **78**, 761.

### ISG15-Activating Enzyme E1, Human

ISG15 (IFN-stimulated gene, 15 kDa) proteins is a UbL (ubiquitin-like protein) that consists of two UB (ubiquitin)-related domains, identical to UbLs conjugated to cellular proteins after IFN $\alpha/\beta$ -stimulation. ISG15-activating enzyme is responsible for the first step in ISG15-protein isopeptide bond formation and is a critical component for the initiation of any *in vitro* conjugation reactions. Purity:  $\geq 98\%$  by SDS-PAGE.

Cat. No. 662076                      25  $\mu$ g                      € 306

Ref.: Yuan, W., et al. 2001. *EMBO. J.* **20**, 362; Hemelaar, J., et al. 2004. *Mol. Cell. Biol.* **24**, 84.

### SUMO-Activating Enzyme E1 (Aos1/Uba2), Human

SUMO (small ubiquitin-related modifier)-activating enzyme is a heterodimer composed of Uba2 and Aos1 polypeptides, which resembles ubiquitin in its structure, its ability to be ligated to other proteins, as well as in the mechanism of ligation. However, SUMOlation doesn't mark proteins for degradation. It mediates ATP-dependent activation of UBL1 (ubiquitin-like 1) and formation of a thiol-ester with a conserved cysteine residue on SAE2.

Purity:  $\geq 98\%$  by SDS-PAGE.

Cat. No. 662074                      25  $\mu$ g                      € 306

### SUMO-Activating Enzyme E1 (Aos1/Uba2), GST-Fusion protein, Human

Cat. No. 662073                      50  $\mu$ g                      € 306

Ref.: Dohmen, R.J., et al. 1995. *J. Biol. Chem.* **270**, 18099; Gong, L., et al. 1999. *FEBS Lett.* **448**, 185; Johnson, E.S., et al. 1997 *EMBO J.* **16**, 5509.

## Now in Stock!

### Forskolin, 7-Deacetyl-7-[O-(N-methylpiperazino)- $\gamma$ -butyryl]-, Dihydrochloride

A chemically-modified forskolin that offers greater stability and water solubility than the parent compound.

Cat. No. 344273                      5 mg                      € 216  
                                                 10 mg                      356



Our Novabiochem® brand is the leading provider of state-of-the-art, innovative building blocks, resins, and solid-supported reagents for peptide, protein, and high-throughput chemical synthesis.

Find out about the sophisticated tools the Novabiochem brand can offer for:

- the conjugation and immobilization of peptides, proteins, carbohydrates, and nucleic acids
- the synthesis of biologically active peptides and small proteins
- the PEGylation of biomolecules
- the labeling of peptides

Request your personal copy of the Novabiochem 2004/05 Catalog at [www.novabiochem.com](http://www.novabiochem.com)

# Novabiochem®



## NEW! Antibodies for Transcription Factors

### Anti-MafA, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to a portion of MafA located near the C-terminal region. Detects the ~48 kDa MafA in mouse. MafA is a transcription factor, which is a glucose-regulated and  $\beta$ -cell-specific activator of the insulin gene.

Cat. No. DR1019      100  $\mu$ g      € 361

### Anti-MafB, Rabbit pAb

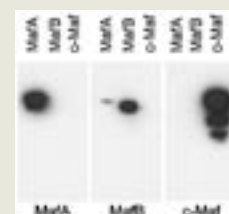
Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to an internal domain of v-maf musculoaponeurotic fibrosarcoma oncogene homolog B. Detects the ~36 kDa MafB in mouse. MafB is a transcription factor involved in hindbrain development and is an inducer of monocytic differentiation.

Cat. No. DR1020      100  $\mu$ g      € 361

### Anti-c-Maf, Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic peptide corresponding to an internal domain of c-maf musculoaponeurotic fibrosarcoma oncogene homolog C. Detects the ~39 kDa c-Maf in mouse. c-Maf plays a role in the regulation of glucagons and is also commonly expressed in multiple myelomas.

Cat. No. DR1021      100  $\mu$ g      € 361



Detection of c-Maf by immunoblotting. Sample: Nuclear extracts (6  $\mu$ g) from HeLa cells transfected with MafA, MafB, or c-Maf expression constructs. Primary antibodies: Anti-MafA, Rabbit pAb (Cat. No. DR1019), Anti-MafB, Rabbit pAb (Cat. No. DR1020), and Anti-c-Maf, Rabbit pAb (Cat. No. DR1021). Each antibody was used at 1:2,000 dilution.

**Note:** These antibodies are suitable for immunoblotting (1:1,000 to 1:10,000), immunohistochemistry (1:1,000 to 1:3,000), gel shift assay (1 to 5  $\mu$ g/20 ml), and chromatin immunoprecipitation (5 to 15  $\mu$ g/10<sup>8</sup> cells).

### NF- $\kappa$ B Antibody Sampler Kit

Each kit contains four separate vials, each containing 20  $\mu$ l rabbit serum: Anti-NF- $\kappa$ B (p50), Rabbit pAb (Cat. No. PC136), Anti-NF- $\kappa$ B (p65), Rabbit pAb (Cat. No. PC137), Anti-c-Rel, Rabbit pAb (Cat. No. PC139), and Anti-I $\kappa$ B $\alpha$ , Rabbit pAb (Cat. No. PC142). Daudi cells may be used as positive control.

Dilution for immunoblotting: 1:500 (chemiluminescence or colorimetric detection)

Antibody	Epitope	Species Reactivity	Applications
Anti-NF- $\kappa$ B (p50), Rabbit pAb	N-terminal region of human NF- $\kappa$ B (p50) protein	Human	GS, IB, IP, ELISA
Anti-NF- $\kappa$ B (p65, RelA), Rabbit pAb	C-terminal region of human NF- $\kappa$ B (p65, RelA) protein	Human, Mouse	GS, IB, IP
Anti-NF- $\kappa$ B (c Rel), Rabbit pAb	C-terminal region of human NF- $\kappa$ B (c Rel) protein	Human	ELISA, GS, IB, IP
Anti-I $\kappa$ B $\alpha$ , Rabbit pAb	C-terminal region of human I $\kappa$ B $\alpha$ protein	Human, Mouse, and Rat	ELISA, IB, IP

ELISA: enzyme-linked immunosorbent assay, GS: gel supershift, IB: immunoblotting, IP: immunoprecipitation

Cat. No. ASK20      1 kit      € 232

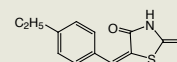
### PhosphoDetect™ Anti-ATF-2 (pThr<sup>71</sup>) Rabbit pAb

Polyclonal IgG, immunoaffinity-purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues surrounding Thr<sup>71</sup> of ATF-2. Detects the ~70 kDa ATF-2 in human, mouse and rat when phosphorylated on Thr<sup>71</sup>. Suitable for immunoblotting (1:1000), immunocytochemistry (1:5000), immunoprecipitation (1:250), and immunohistochemistry on free floating sections (1:100), and paraffin sections (1:100).

Cat. No. ST1075      50  $\mu$ l      € 202

### c-Myc Inhibitor

A cell-permeable thiazolidinone compound that specifically inhibits the c-Myc-Max interaction, thereby preventing the transactivation of c-Myc target gene expression. Shown to inhibit tumor cell growth in a c-Myc-dependent manner both *in vitro* and *in vivo* (effective concentration: 64  $\mu$ M using c-Myc transfected Rat1a fibroblasts).



*Purity:  $\geq$ 95% by HPLC (sum of two isomers). M.W. 249.4.*

Cat. No. 475956      10 mg      € 106

Ref.: Yin, X., et al. 2003. *Oncogene* 22, 6151.

## NEW! Oxidative Stress Research Tools

### Mn-cpx 3

A cell-permeable manganese complex of 7-hydroxyflavone that acts as a superoxide dismutase (SOD) mimetic and a neuroprotective agent against free radical-induced damage. Shown to prevent the reduction of NBT by  $O_2^{\bullet -}$  ( $IC_{50} = 1.18 \mu M$ ) and exhibit ~10 times higher potency than Trolox (Cat. No. 648471) in inhibiting lipid peroxidation ( $IC_{50} = 6.15 \mu M$ ). *Purity:  $\geq 98\%$  by elemental analysis. M.W. 602.3.*

Cat. No. 475867      25 mg      € 112

Ref.: Vajragupta, O., et al. 2003. *Bioorg. Med. Chem.* 11, 2329.

### MitoPBN

A nitron antioxidant derived from IBTP (Cat. No. 401009) that is shown to block superoxide-induced lipid peroxidation in rat liver mitochondrial preparations without disrupting normal mitochondrial functions when used at concentrations below  $25 \mu M$ . It selectively and rapidly traps carbon-centered secondary radicals, but is unreactive towards superoxide anion ( $O_2^{\bullet -}$ ) or alkylperoxide radicals ( $ROO^{\bullet}$ ). *Purity:  $\geq 95\%$  by HPLC. M.W. 590.5.*

Cat. No. 475822      10 mg      € 177

Ref.: Murphy, M.P., et al. 2003. *J. Biol. Chem.* 278, 48534. Smith, R.A.J., et al. 2003. *Biochem. Soc. Trans* 31, 1295.

### Hydrogen Peroxide Probe, Fluorogenic (Pentafluorobenzenesulfonyl fluorescein)

A monosulfonated non-fluorescent fluorescein ester that acts as a sensitive, selective, non-oxidative mechanism based sensor for hydrogen peroxide ( $H_2O_2$ ) with a detection limit of 46 pmol at  $25^\circ C$ . Gets converted to fluorescein upon perhydrolysis with  $H_2O_2$  (relative quantum efficiency = 0.003). Also detects superoxide ( $O_2^{\bullet -}$ ) and displays selectivity over hydroxyl radicals ( $OH^{\bullet}$ ), peroxynitrite ( $ONOO^-$ ), tert-butylhydroperoxide (t-BuOOH) and nitric oxide ( $NO^{\bullet}$ ). Undergoes slow hydrolysis in HEPES buffer at the rate of 1.1% per hour at  $25^\circ C$ , pH = 7.4.

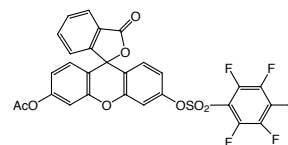
*Purity:  $\geq 98\%$  by HPLC. M.W. 562.4.*

Cat. No. 386793      10 mg      € 104

Ref.: Maeda, H., et al. 2004. *Angew. Chem. Int. Ed.* 43, 2389.

### Hydrogen Peroxide Probe, Fluorogenic, Cell-permeable (Acetyl, pentafluorobenzenesulfonyl fluorescein)

A cell-permeable acetylated version of the Hydrogen Peroxide Probe, Fluorogenic (Cat. No. 386793) that is useful for the intracellular measurements of  $H_2O_2$ . Undergoes deacetylation by the cellular esterases to the membrane impermeable, non-fluorescent form (Cat. No. 386793). *Purity:  $\geq 98\%$  by HPLC. M.W. 604.5.*



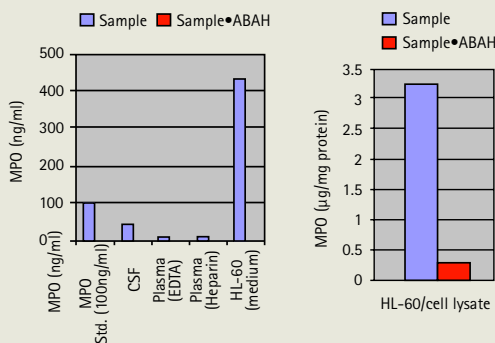
Cat. No. 386794      5 mg      € 104

Ref.: Maeda, H., et al. 2004. *Angew. Chem. Int. Ed.* 43, 2389.

## Introducing...

### InnoZyme™ Myeloperoxidase Activity Kit

A specific and sensitive kit designed to measure human myeloperoxidase (MPO) activity in cell lysates and biological samples and to screen MPO inhibitors. The assay uses a polyclonal antibody specific for human MPO immobilized on a 96-well plate to specifically capture the enzyme. Activity of captured MPO is measured using a detection reagent that includes TMB and hydrogen peroxide. Following color development, the reaction is stopped with sulfuric acid and the absorbance of the oxidized TMB is detected at 450 nm. Detection range: 5 - 100 ng/ml.



Myeloperoxidase (MPO) activity in biological samples in the absence and presence of 20  $\mu M$  MPO inhibitor, 4-aminobenzoyl hydrazide (ABAH). Cells were cultured in RPMI 1600 medium supplemented with 10% fetal calf serum and harvested at 80-90% confluency. Cell lysates were prepared by treating cell pellets with CytoBuster™ Protein Extraction Reagent (Cat. No. 71009).

Cat. No. CBA024      1 kit      € 483

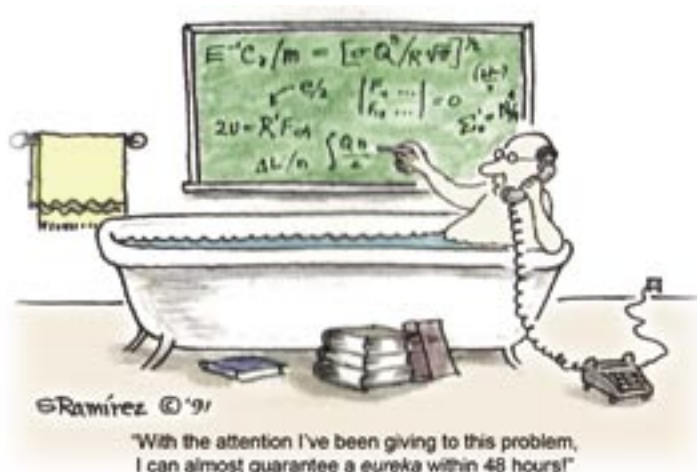
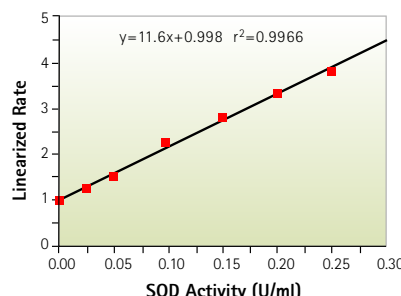
## Superoxide Dismutase Assay Kit II

A sensitive spectro-  
photometric assay

kit for measur-  
ing the activity  
of all three types  
(Cu/Zn, Mn, and  
Fe) of superoxide  
dismutase (SOD)

in plasma, serum,  
erythrocyte lysates,

tissue homogenates, and cell lysates. This kit uses a tetra-  
zolium salt for the detection of superoxide radicals. Assay  
range: 0.025 - 0.25 units per ml SOD. Each kit contains  
reagents sufficient for up to 100 tests.



Cat. No. 574601

1 kit

€ 459

## NEW! Technical Tips Section

### How much of an inhibitor or stimulator should one inject into an animal?

There is no simple answer to this question. One must optimize the dose empirically by performing a few preliminary experiments. First determine if the compound in question is cell-permeable. Also, survey the literature for any reported  $IC_{50}$ ,  $ED_{50}$ , or  $EC_{50}$  values. One may follow the sample calculation given below as a general guide:

H-89, dihydrochloride, a cell-permeable protein kinase A inhibitor, has an  $IC_{50}$  value of 48 nM. It has a molecular weight of 519.3. Hence, 240 to 480 nM range of H-89 is sufficient to cause maximal inactivation of protein kinase A. To use it *in vivo* we have to make a few assumptions. If a rat weighs about 200 g and we assume that 70% of its body weight is water, the volume of distribution will be approximately 140 ml. In this case 240 nM = 240 nmoles/liter = 124.63 mg/liter. Because the volume of distribution is about 140 ml,  $124.63 \times 0.140 = 17.45$  mg would be the required amount for injection into the rat. It is important to note that the drug distribution will vary depending on the mode of injection (intravenous, intramuscular, or intraperitoneal), bioavailability, half-life, rates of hepatic and renal clearance, binding to proteins, and tissue-specific distribution and accumulation. The specific tissue uptake may also be limited in whole organs or tissues as compared to isolated cell preparations. In whole animal studies, sometimes a loading dose is required to achieve the target concentration. This may then be followed by a sustained infusion to maintain the drug level in the blood. One must always exercise caution and not overdose the animal.

Prices and availability are subject to change. ©Copyright 2005 Merck Biosciences, an affiliate of Merck KGaA, Darmstadt, Germany. All rights reserved. Each product is sold with a limited warranty which is provided with each purchase. Each product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. Merck Biosciences products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Merck Biosciences.

Calbiochem®, Novagen®, His •Tag®, and ZWITTERGENT® are registered trademarks of EMD Biosciences, Inc. in the United States and certain other jurisdictions. Novabiochem® is a registered trademark of Merck Biosciences AG. ATP-Binders™, CytoBuster™, D-Tube™, InnoCyte™, InnoZyme™, K-LISA™, NoShift™, NucBuster™, PhosphoSafe™ and ProteoEnrich™ are trademarks of EMD Biosciences, Inc. ProteoExtract® is a registered trademark of Merck KGaA, Darmstadt, Germany. TRITON® is a registered trademark of Dow Chemical Company. TWEEN® is a registered trademark of ICI Americas Inc. Coomassie™ is a trademark of Imperial Chemical Industries plc. Alexa Fluor®, Pro-Q®, SYPRO®, and SYTOX® are registered trademarks of Molecular Probes, Inc. QuickZyme™ is a trademark of TNO.

For more information or to order Calbiochem products, contact  
Merck Biosciences GmbH  
Postfach 1167, 65796 Bad Soden

**Germany**

*Orders*

Tel 0800 6931 000

Fax 0800 6236 100

*Technical Support:*

Tel 0800 100 3496

E-mail [techservice@merckbiosciences.de](mailto:techservice@merckbiosciences.de)

**Republic of Ireland**

Tel 1800 409445

*Visit our website*

[www.merckbiosciences.de](http://www.merckbiosciences.de)

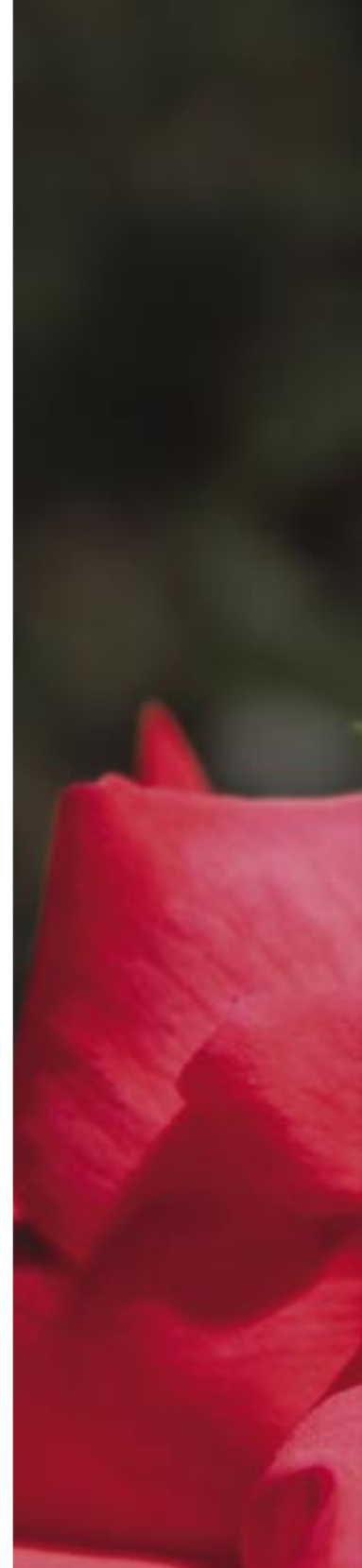
You can also order Calbiochem products through  
our distribution partner, VWR International GmbH

Tel 0180 570 2000

Web [www.vwr.com](http://www.vwr.com)

Calbiochem, Novabiochem, and Novagen are brands of Merck  
Biosciences, an affiliate of Merck KGaA, Darmstadt, Germany

Printed in the USA



BIOL-31.1EURO