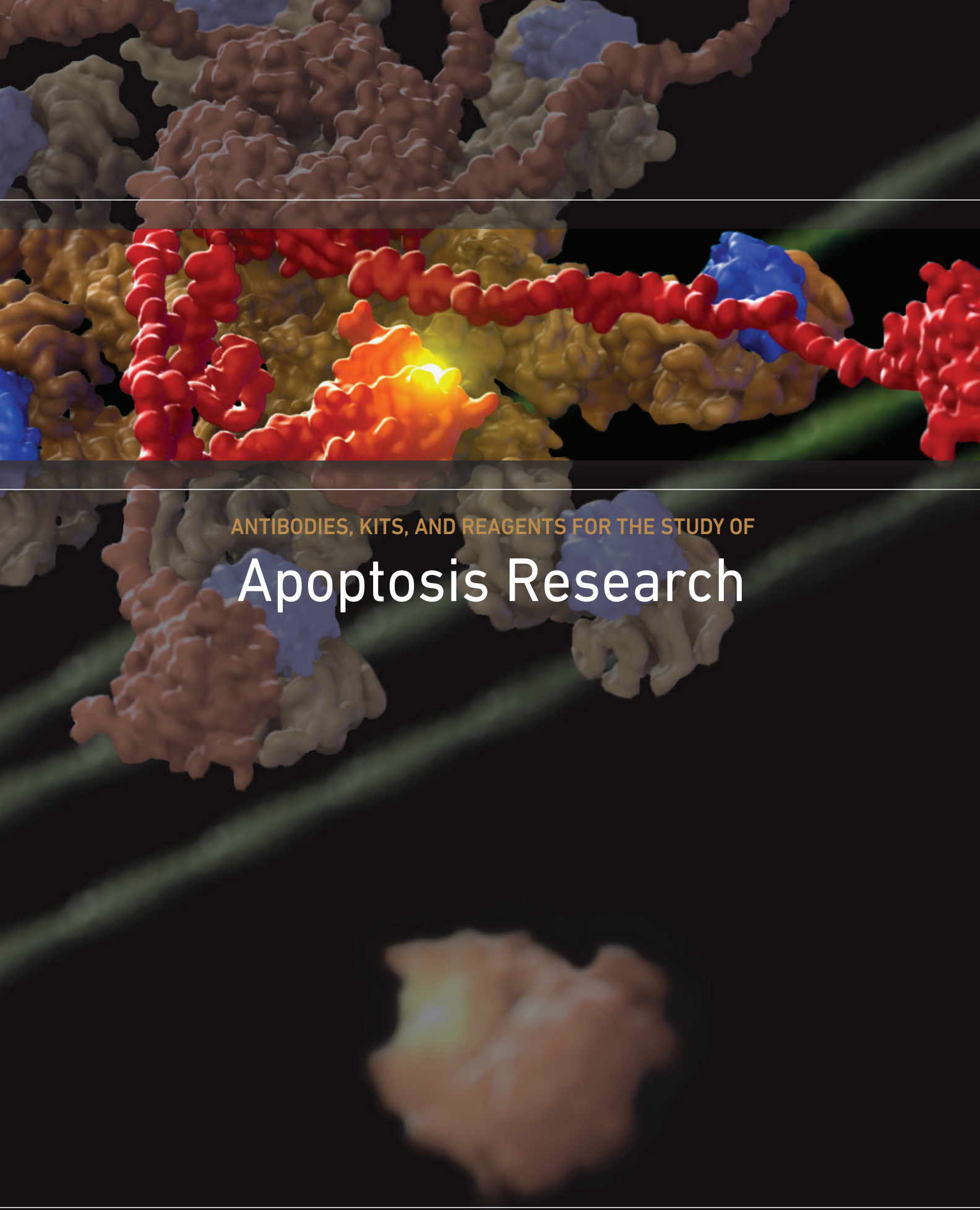
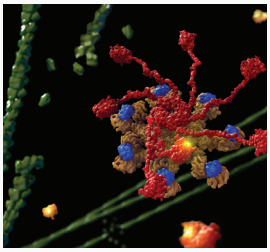


ANTIBODIES, KITS, AND REAGENTS FOR THE STUDY OF

Apoptosis Research

Cell Signaling TECHNOLOGY®

COVER IMAGE: Cytochrome c release from the mitochondrion during apoptosis leads to amplification of the caspase signaling cascade via formation of the apoptosome. A radially-symmetric structure, the apoptosome assembles upon binding of cytochrome c (blue) to Apaf-1 (beige), followed by recruitment of caspase-9 (red). Once assembled, the apoptosome's activated caspase-9 cleaves and activates cytosolic caspase-3 (red, floating) and rapidly amplifies the caspase cascade. This leads to the destruction of numerous intracellular targets by caspase-3 including the actin cytoskeleton (green).



Contents

4 Tools and Targets

6 Intrinsic Pathway Tools

8 Extrinsic Pathway Tools

10 Apoptosis and Cancer

14 Validation and Support

Shuji, Scientist, has been with CST since 2008.

Apoptosis

Apoptosis is a tightly controlled pattern of cell death characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation. The central regulators of apoptosis are the caspases, a family of cysteine proteases that fall into two broad categories. The "initiator" caspases-2, -8, -9, -10, and -12 are closely coupled to upstream, pro-apoptotic signals and act by cleaving and activating the downstream "executioner" caspases-3, -6, and -7 that modify proteins ultimately responsible for programmed cell death. Targets such as PARP and lamin A/C are cleaved by executioner caspases and serve as markers of apoptosis.

There are two primary apoptotic pathways that lead to caspase activation: the intrinsic and extrinsic pathways. The intrinsic pathway is activated by cell stress, DNA damage, developmental cues, and withdrawal of survival factors. This pathway is regulated by the Bcl-2 family of proteins, which consists of pro-apoptotic (Bax, Bak, Bad, Bid, Puma, Bim, and Noxa) and anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, Mcl-1) factors. Bcl-2 family members localize to the mitochondria where they control mitochondrial permeability, release of cytochrome c, and activation of initiator caspase-9 followed by executioner caspase-3. Intrinsic apoptosis can be inhibited through signaling pathways that promote survival, such as the PI3K/Akt and MAPK pathways.

Caspases can also be activated through the extrinsic or death receptor pathway. The death receptors consist of members of the TNFR family (TNFR1/2, Fas, and DR3/4/5) and their associated ligands (TNF-α, FasL, TRAIL, TWEAK). Ligand binding induces receptor activation, leading to signaling through the adaptor proteins FADD and TRADD to activate the initiator caspase-8 and the executioner caspase-3. The death receptor pathway can also lead to cell survival through TNFR2-mediated signaling to NF-κB, which induces expression of pro-survival genes, Bcl-2 and FLIP.

Selected Reviews: Dickens, LS, Powley, IR, Hughes, MA, et al. (2012) *Exp. Cell Res.* 318, 1269–1277. | Favaloro, B, Allocati, N, Graziano, V, et al. (2012) *Aging* 4, 330–349. | McIlwain, DR, Berger, T, and Mak, TW. (2013) *Cold Spring Harb. Perspect. Biol.* 5, a008656. | Shamas-Din, A, Kale, J, Leber B, et al. (2013) *Cold Spring Harb. Perspect. Biol.* 5, a008714.

A Trusted Research Partner

Cell Signaling Technology (CST) strives to be your research partner for the study of apoptosis. As scientists we understand the importance of using antibodies that consistently work each and every time. Our highly specific antibodies are directed against the most relevant targets in apoptosis and are painstakingly validated to work in their recommended applications, so you can feel confident in your results. In addition, we provide siRNA, chemical modulators, control cell extracts, and kits—all validated using the same rigorous quality standards—giving you the tools you need for every step of the experimental process. Optimal antibody dilutions and recommended buffers are predetermined for you, saving you the time, sample, and trouble of additional optimization steps. Protocols and troubleshooting guides for commonly used applications are available on our website to help you get reliable results in the shortest amount of time. If you experience a problem in the lab, the same expert scientist who produced and validated your antibody will respond to your email or phone call and help you, sharing their bench experience and data from their notebooks.

Products and Tools

for Apoptosis Research

CST offers the highest possible quality antibodies and reagents for each stage of the experimental process.

Primary Antibodies

CST offers a broad range of highly specific primary antibodies to key targets in apoptosis signaling pathways. Currently, we offer more than 300 primary antibodies against over 150 protein targets, including phosphorylation site and cleavage-specific antibodies. Our portfolio is constantly expanding, so please check our website frequently for a complete, up-to-date product list.

Antibody Sampler Kits

Sampler kits allow for the simultaneous analysis of multiple nodes in a pathway of interest or modification sites within a protein of interest.

Assay Kits

Assess apoptosis using a CST assay kit, scale up your analysis in a 96-well format using PathScan® ELISA Kits (384-well plates are also available on a custom basis), or monitor multiple pathway nodes in parallel using sandwich assays in a slide-based array.

SignalSilence siRNA

Rigorously validated SignalSilence® siRNAs can be used to selectively reduce the expression of a protein of interest.

Chemical Modulators

Treat cells with a chemical modulator to induce apoptosis and examine effects on downstream signaling.

Experimental Controls

Control cell extracts, control proteins, blocking peptides, and isotype controls can help verify antibody specificity, critical for accurate data analysis.

Companion Products

Secondary antibodies, loading controls, buffers, dyes, chemical modulators, detection reagents, protease inhibitors, and peptide standards are available to support your protocol.

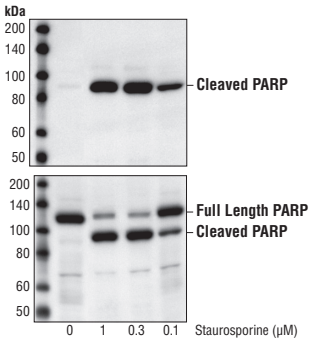
We've got it covered

A1/Bfl-1
Acinus
Phospho-Ack1 (Tyr284)
AIF
Alix
AP-2α, -2β, -2γ
Phospho-AP2M1 (Thr156)
Apaf-1
APR3
Aven
Bad
Phospho-Bad (Ser112), (Ser136), (Ser155)
Bak
BAP31
Bax
Bcl-2
Phospho-Bcl-2 (Thr56), (Ser70)
Bcl-w
Bcl-xL
BCL2L10
BID
Bik
Bim
Phospho-Bim (Ser55), (Ser69), (Ser77)
BIRC6
Bmf
BNIP3
BNIP3L
Phospho-BNIP3L (Ser62)
Bok
c-IAP1
c-IAP2
Caspase-1
Cleaved Caspase-1 (Asp297)
Caspase-2
Caspase-3
Cleaved Caspase-3 (Asp175)
Caspase-4
Caspase-5
Caspase-6
Cleaved Caspase-6 (Asp162)
Caspase-7
Cleaved Caspase-7 (Asp198)
Caspase-8
Cleaved Caspase-8 (Asp374), (Asp384), (Asp391)
Caspase-9
Phospho-Caspase-9 (Thr125), (Asp315), (Asp330), (Asp353)
Caspase-12
Caspase-14
Caspase Cleavage Motif
Cleaved Caspase Substrate
CRADD/RAIDD
Cytochrome c
Acetyl-Cytochrome c (Lys8)
DAP1
DAP3
DAPK1
DAPK3/ZIPK
Daxx
Cleaved Drosophila Dcp-1 (Asp216)
DcR1
DcR2
DcR3
DFF45/DFF35
Cleaved DFF45 (Asp224)
DID01
DR3
DR4
DR5
Phospho-DR6 (Ser562)
DRAK2

Tools to Support Your Apoptosis Workflow

Apoptotic Stimuli

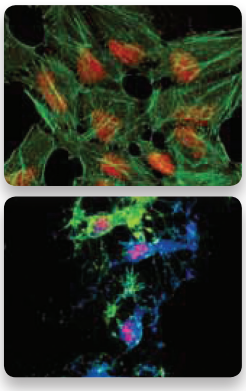
Treat cells with a chemical modulator to induce apoptosis



Staurosporine #9953: WB analysis of extracts from HeLa cells, untreated or Staurosporine-treated (3 hr), showing PARP cleavage as evidence of induction of apoptosis, using Cleaved PARP Antibody #9541 (upper) or PARP Antibody #9542 (lower).

Cellular Readouts

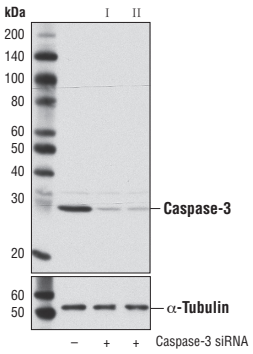
Antibodies to key targets as cellular readouts for apoptosis



Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Alexa Fluor® 647 Conjugate) #9602: Confocal IF analysis of HeLa cells, untreated (upper) or treated with Staurosporine #9953 (1 μM, 4 hr; lower), using #9602 (blue pseudocolor). Actin filaments were labeled with Alexa Fluor® 488 Phalloidin #8878 (green). Red = Propidium Iodide (PI)/RNase Staining Solution #4087.

Experimental Controls

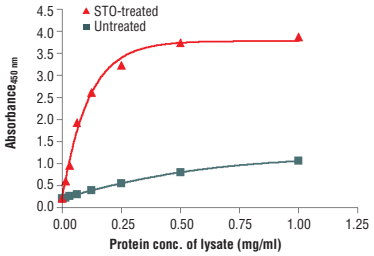
Control cell extracts and siRNAs to confirm target specificity

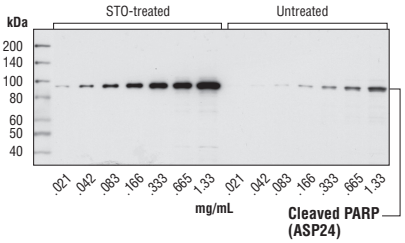


SignalSilence® Caspase-3 siRNA I (Mouse Specific) #6488 and SignalSilence® Caspase-3 siRNA II (Mouse Specific) #6501: WB analysis of extracts from NIH/3T3 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #6488 (+), or #6501 (+), using Caspase-3 (8G10) Rabbit mAb #9665 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The caspase-3 (8G10) Rabbit mAb confirms silencing of Caspase-3 expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.

Assays Kits

Apoptosis Detection, ELISA, and Multi-Target Array Kits for experimental analysis





PathScan® Cleaved PARP (Asp214) Sandwich ELISA Kit #7262: The relationship between protein concentration of lysates from untreated and staurosporine (STO) treated HeLa cells and the absorbance at 450 nm is shown. HeLa cells (80% confluent) were treated with staurosporine (1 μM) for 3 hours. Absorbance at 450 nm is shown in the top figure, while the corresponding WB using Cleaved PARP Antibody #9548 is shown in the bottom figure.

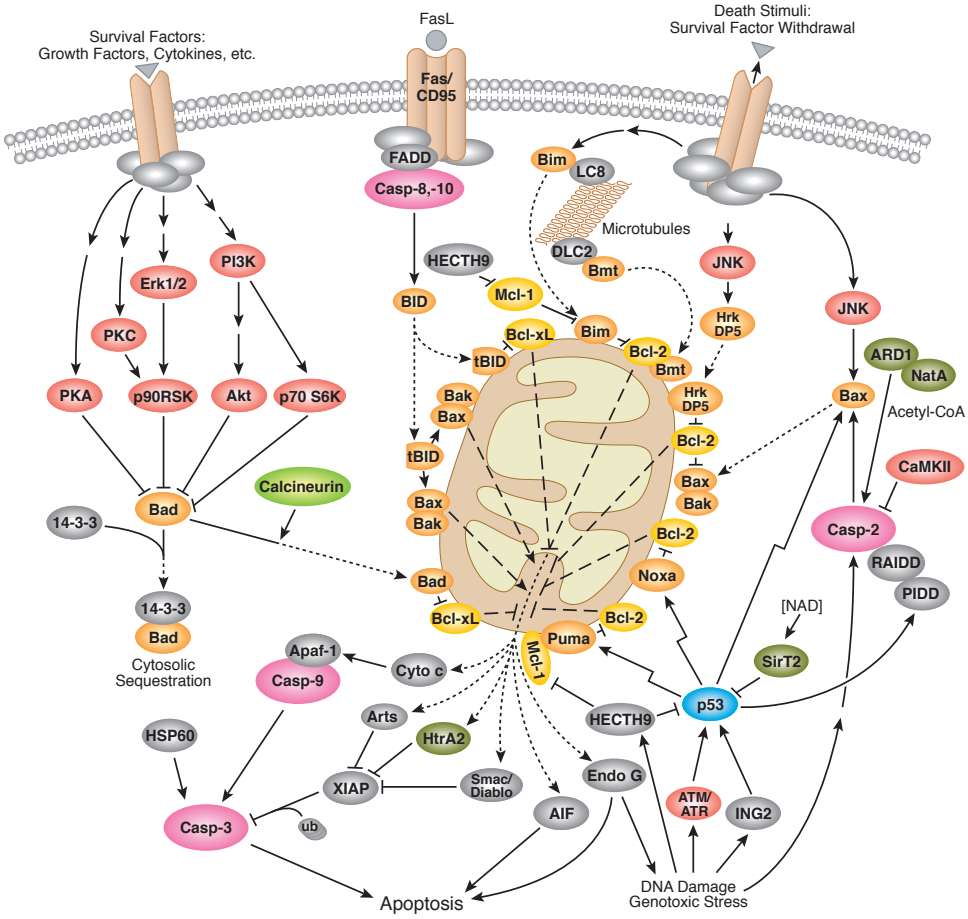
Our total and modification-specific antibody collection provides comprehensive coverage of the apoptosis pathways.

eIF4G2/p97
Endonuclease G
FADD
Phospho-FADD (Ser191), (Ser194)
FAF1
FAIM
Fas
FasL
FLIP
α-Fodrin
Cleaved α-Fodrin (Asp1185)
Granzyme A
Granzyme B
HIPK2
HtrA2/Omi
Drosophila ICE
Cleaved Drosophila ICE (Asp230)
Lamin A/C
Phospho-Lamin A/C (Ser22)
Cleaved Lamin A (Asp230), (Small Subunit)
Lamin B1
Lamin B2
LAP2α
Livin
Mad-1
Maspin
Max
Mcl-1
Phospho-Mcl-1 (Ser64), (Ser159/Thr163)
Mst1
Mst2
Phospho-Mst1 (Thr183)/Mst2 (Thr180)
Mst3
Mst3b
Mst4
c-Myc
Phospho-c-Myc (Thr58/Ser62)
N-Myc
PAR-4
Phospho-PAR-4 (Thr163)
PARP
Cleaved PARP (Asp214)
PDCD4
PEA-15
Phospho-PEA-15 (Ser104)
Perforin
PHLDA3
Puma
Siva-1
Smac/Diablo
Survivin
Phospho-Survivin (Thr34)
TANK
TAX1BP1
Thymidine Phosphorylase/ECGF1
TMS1
TNF-R2
TP/ECGF1
TRADD
TRAF1
TRAF2
TRAF3
TRAF6
TRAIL
VDAC1
VDAC2
WWOX
XAF1
XIAP

Intrinsic Apoptosis:

Bcl-2 Proteins

Broad range of tools to help you examine apoptosis from beginning to end

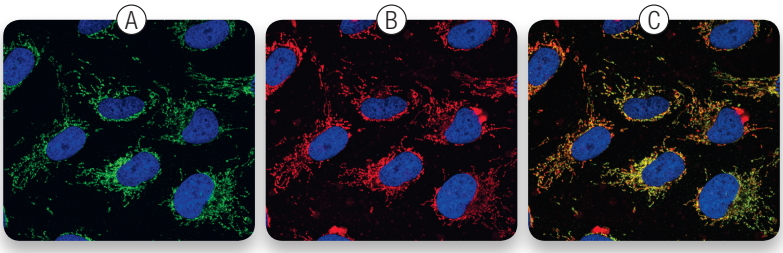


The Bcl-2 family of proteins regulates apoptosis by controlling mitochondrial permeability. The anti-apoptotic proteins Bcl-2 and Bcl-xL reside in the outer mitochondrial wall and inhibit cytochrome c release. The pro-apoptotic Bcl-2 proteins Bad, BID, Bax, and Bim may reside in the cytosol but translocate to the mitochondria following death signaling, where they promote the release of cytochrome c. Bad translocates to mitochondria and forms a pro-apoptotic complex with Bcl-xL. This translocation is inhibited by survival factors that induce the phosphorylation of Bad, leading to its cytosolic sequestration. Cytosolic BID is cleaved by caspase-8 following signaling through Fas; its active fragment (tBID) translocates to mitochondria. Bax and Bim translocate to mitochondria in response to death stimuli, including survival factor withdrawal. Activated following DNA damage, p53 induces the transcription of Bax, Noxa, and Puma. Upon release from mitochondria, cytochrome c binds to Apaf-1 and forms an activation complex with caspase-9. Although the mechanism(s) regulating mitochondrial permeability and the release of cytochrome c during apoptosis are not fully understood, Bcl-xL, Bcl-2, and Bax may influence the voltage-dependent anion channel (VDAC), which may play a role in regulating cytochrome c release. HECTH9 is a DNA damage-activated E3 ubiquitin ligase for p53, and Mcl-1 is an anti-apoptotic member of the Bcl-2 family.

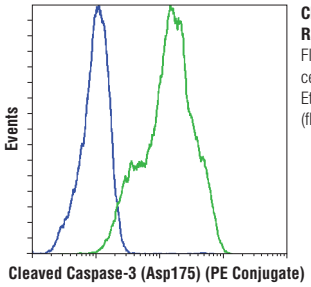
Select Reviews

Brenner, D and Mak, TW. (2009) *Curr. Opin. Cell Biol.* 21, 871–877. | Chalah, A and Khosravi-Far, R. (2008) *Adv. Exp. Med. Biol.* 615, 25–45. | Lindsay, J, Esposti, MD, and Gilmore, AP. (2011) *Biochim. Biophys. Acta.* 1813, 532–539. | Ola, MS, Nawaz, M, and Ahsan, H. (2011) *Mol. Cell. Biochem.* 351, 41–58. | Pradelli, LA, Bénéteau, M, and Ricci, JE. (2010) *Cell. Mol. Life Sci.* 67, 1589–1597. | Rong, Y and Distelhorst, CW. (2008) *Annu. Rev. Physiol.* 70, 73–91. | Speidel, D. (2010) *Trends Cell Biol.* 20, 14–24. | Suen, DF, Norris, KL, and Youle, RJ. (2008) *Genes Dev.* 22, 1577–1590.

Antibodies to key targets as cellular readouts

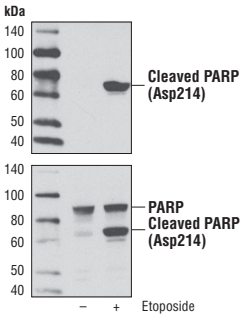


Bak (D4E4) Rabbit mAb #12105: Confocal IF analysis of OVCAR8 cells showing colocalization with mitochondria using #12105 (green) **(A)** and MitoTracker® Red CMXRos (Red) **(B)**. Merged image **(C)**. Blue pseudocolor = DRAQ® #4084 (fluorescent DNA dye).



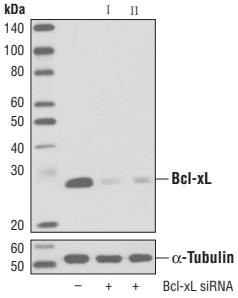
Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (PE Conjugate) #12768: Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with Etoposide #2200 (green), using #12768 (fluorescent DNA dye).

Chemical modulators to induce apoptosis



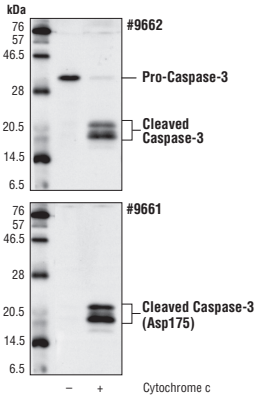
Etoposide #2200: WB analysis of extracts from Jurkat cells, untreated (-) or Etoposide-treated (25 µM, overnight; +), using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb #5625 (upper) or total PARP Antibody #9542 (lower).

SignalSilence® siRNA for knockdown studies



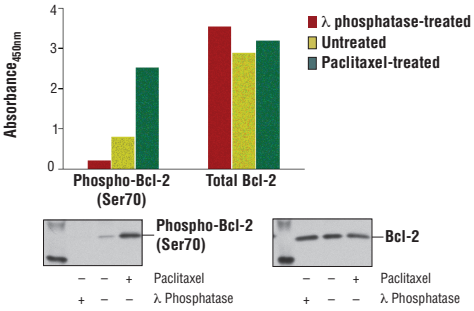
SignalSilence® Bcl-xL siRNA I #6362: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #6362 (+), or SignalSilence® Bcl-xL siRNA II #6363 (+), using Bcl-xL (54H6) Rabbit mAb #2764 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The Bcl-xL (54H6) Rabbit mAb confirms silencing of Bcl-xL expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.

Experimental controls for accurate data analysis



Caspase-3 Control Cell Extracts #9663: WB analysis of Jurkat cell extracts untreated or treated with cytochrome c in vitro, showing full length and/or cleaved caspase-3 (upper) and cleaved caspase-3 Asp175 (lower), using Caspase-3 Antibody #9662 and Cleaved Caspase-3 (Asp175) Antibody #9661.

PathScan® ELISA kits for experimental analysis



PathScan® Phospho-Bcl-2 (Ser70) Sandwich ELISA Kit #11874: Treatment of Jurkat cells with Paclitaxel stimulates phosphorylation of Bcl-2 at Ser70, as detected by #11874, but does not affect the levels of total Bcl-2 detected by PathScan® Total Bcl-2 Sandwich ELISA Kit #12030. Jurkat cells were untreated or treated with λ phosphatase or Paclitaxel #9807 (1 mM, 20 hr, 37°C). The absorbance readings at 450 nm are shown in the upper figure, while the corresponding WBs (lower) using Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb #2827 (left) or Bcl-2 (D55G8) Rabbit mAb (Human Specific) #4223 (right) are shown.

Pro-Apoptosis Bcl-2 Family Antibody Sampler Kit #9942

Antibody sampler kits offer a convenient and economical means for the analysis of multiple nodes within a pathway of interest. For a complete listing of our Antibody Sampler Kits:

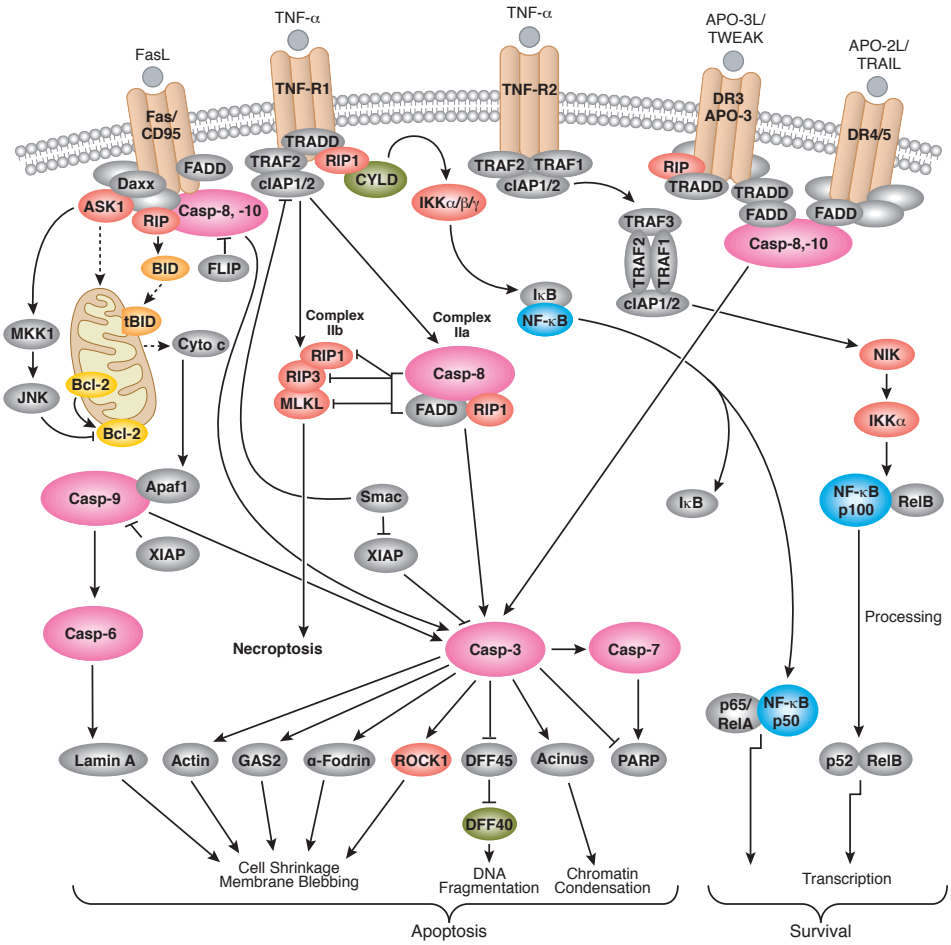
www.cellsignal.com/abkits

Kit Includes:
Bad (D24A9) Rabbit mAb #9239, Phospho-Bad (Ser112) (40A9) Rabbit mAb #5284, Bax (D2E11) Rabbit mAb #5023, Bik Antibody #4592, Bim (C34C5) Rabbit mAb #2933, BID Antibody (Human Specific) #2002, Bak (D4E4) Rabbit mAb #12105, Puma (D30C10) Antibody #12450, Anti-rabbit IgG, HRP-linked Antibody #7074

Extrinsic Apoptosis:

Death Receptors

Antibodies and reagents to support every step of your protocol

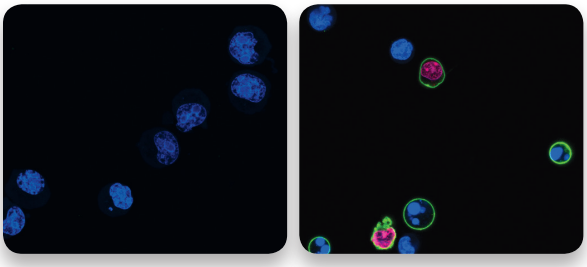


Apoptosis can be induced through the activation of death receptors including Fas, TNFαR, DR3, DR4, and DR5 by their respective ligands. Death receptor ligands characteristically initiate signaling via receptor oligomerization, which in turn results in the recruitment of specialized adaptor proteins and activation of caspase cascades. Binding of FasL induces Fas trimerization, which recruits initiator caspase-8 via the adaptor protein FADD. Caspase-8 then oligomerizes and is activated via autocatalysis. Activated caspase-8 stimulates apoptosis via two parallel cascades: it can directly cleave and activate caspase-3, or alternatively, it can cleave Bid, a pro-apoptotic Bcl-2 family protein. Truncated BID (tBID) translocates to mitochondria, inducing cytochrome c release, which sequentially activates caspase-9 and -3. TNF-α and DR-3L can deliver pro- or anti-apoptotic signals. TNFαR and DR3 promote apoptosis via the adaptor proteins TRADD/FADD and the activation of caspase-8. Interaction of TNF-α with TNFαR may activate the NF-κB pathway via NIK/IKK. The activation of NF-κB induces the expression of pro-survival genes including Bcl-2 and FLIP, the latter can directly inhibit the activation of caspase-8. FasL and TNF-α may also activate JNK via ASK1/MKK7. Activation of JNK may lead to the inhibition of Bcl-2 by phosphorylation. In the absence of caspase activation, stimulation of death receptors can lead to the activation of an alternative programmed cell death pathway termed necroptosis.

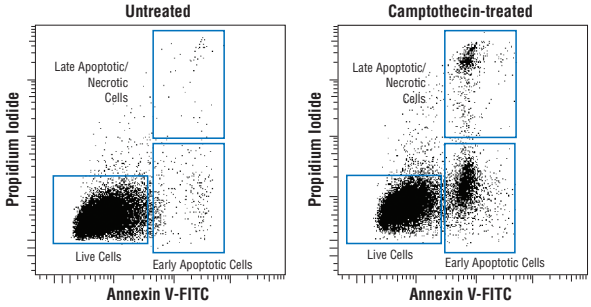
Select Reviews

Declercq, W, Vanden Berghe, T, and Vandenabeele, P. (2009) *Cell* 138, 229–232. | Fuchs, Y and Steller H. (2011) *Cell* 147, 742–758. | Kantari, C and Walczak, H. (2011) *Biochim. Biophys. Acta.* 1813, 558–563. | Kaufmann, T, Strasser, A, and Jost, P.J. (2012) *Cell Death Differ.* 19, 42–50. | Lavrik, IN and Krammer, PH. (2012) *Cell Death Differ.* 19, 36–41. | Van Herreweghe, F, Festjens, N, Declercq, W, and Vandenabeele, P. (2010) *Cell. Mol. Life Sci.* 67, 1567–1579. | Wajant, H and Scheurich, P. (2011) *FEBS J.* 278, 862–876.

Assay kits to measure key cellular readouts

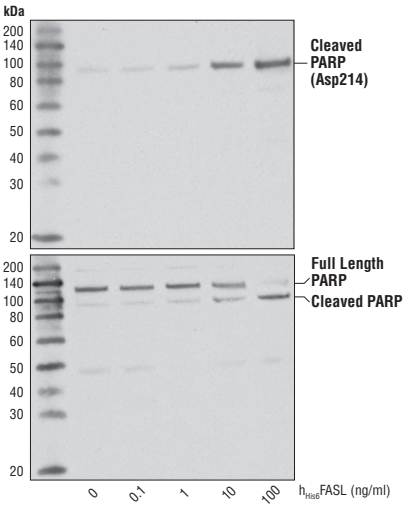


Annexin V-FITC Early Apoptosis Detection Kit #6592: Confocal IF analysis of live Jurkat cells, untreated (left) or camptothecin-treated (right), using Annexin V-FITC Conjugate (green). Red = Propidium Iodide (fluorescent DNA dye). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



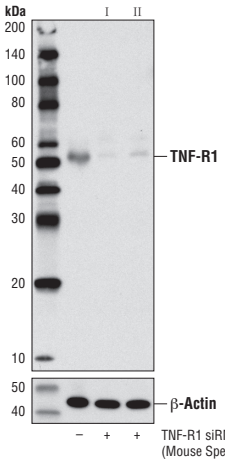
Flow cytometric analysis of Jurkat cells untreated (left) or treated with camptothecin (10 mM, 4 hr; right) using #6592.

Chemical modulators to induce apoptosis



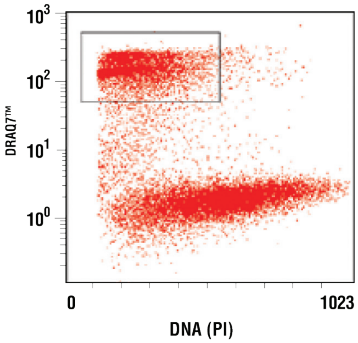
Human $_{His6}$Fas Ligand/TNFSF6 (h_{His6}FasL) #5452: Western blot analysis of extracts from Jurkat cells, untreated or treated with h_{His6}FasL for 3 hr, using Cleaved PARP (Asp214) Antibody (Human Specific) #9541 (upper) or PARP Antibody #9542 (lower).

SignalSilence® siRNA for knockdown studies



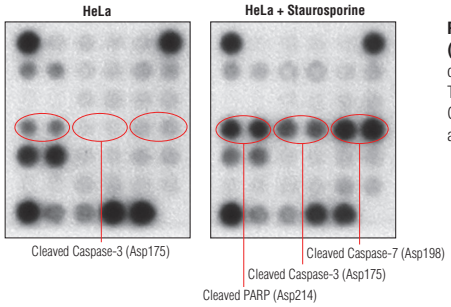
SignalSilence® TNF-R1 siRNA I (Mouse Specific) #13369: WB analysis of extracts from L929 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® TNF-R1 siRNA I (Mouse Specific) (+) or SignalSilence® TNF-R1 siRNA II (Mouse Specific) #13570 (+), using TNF-R1 (D3I7K) Rabbit mAb (Rodent Specific) #13377 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The TNF-R1 (D3I7K) Rabbit mAb (Rodent Specific) confirms silencing of TNF-R1 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

Companion products to support your work



DRAQ7™ #7406: FC analysis of unfixed Jurkat cells treated with Staurosporine #9953. Gated population represents DRAQ7™-positive apoptotic cells.

PathScan® Array kits for multiplex analysis



PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Chemiluminescent Readout) #12856: HeLa cells were grown to 90% confluency and then either untreated (left panel) or treated with Human Tumor Necrosis Factor-α (hTNF-α) #8902 (100 ng/ml, 20 min; right panel). Cell extracts were prepared and analyzed using #12856. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

Death Receptor Antibody Sampler Kit #8356

Explore our antibody sampler kits—convenient and economical ways to assay several nodes in a common signaling pathway. Visit our website to find over 15 sampler kits available for apoptosis research.

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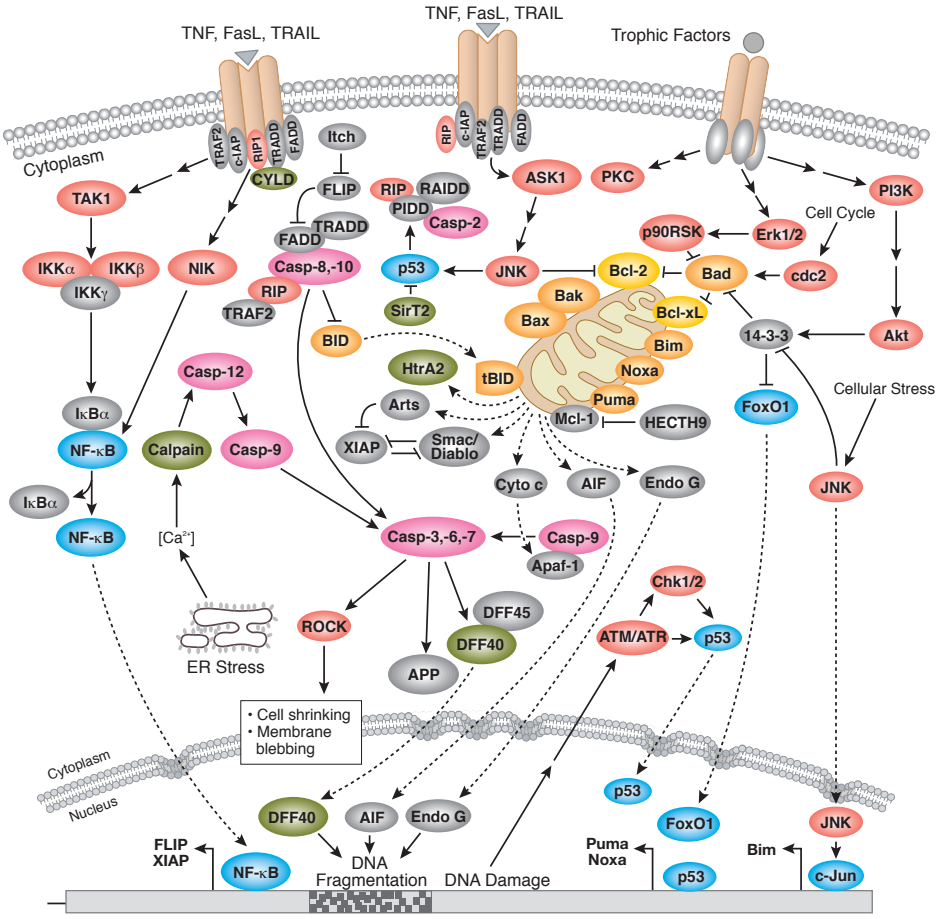
Kit Includes:
Fas (C18C12) Rabbit mAb #4233, TNF-R1 (C25C1) Rabbit mAb #3736, TNF-R2 Antibody #3727, DR3 Antibody #3254, DR5 (D4E9) XP® Rabbit mAb #8074, FADD Antibody (Human Specific) #2782, TRADD (7G8) Rabbit mAb #3684, DcR3 Antibody #4758, RIP (D94C12) XP® Rabbit mAb #3493, Anti-rabbit IgG, HRP-linked Antibody #7074

Survival Signaling

in cancer

Comprehensive pathway coverage to simplify your experimental design



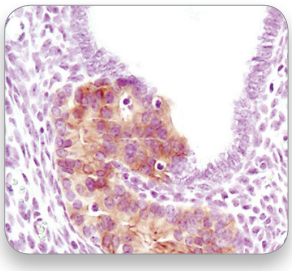
Resisting apoptosis is the one of the six hallmark traits of cancer. Cancer cells have developed unique strategies for cell survival that involve both inhibition of pro-apoptotic factors combined with activation of anti-apoptotic factors. One of the primary pathways involved in cell survival is PI3K/Akt. Akt phosphorylates and inhibits several pro-apoptotic factors such as Bim, Bax, Bad, and FoxO1. Akt also activates the inhibitor of apoptosis protein XIAP and blocks p53-mediated apoptosis through stabilization of MDM2. Mutations that result in constitutive signaling through this pathway provide distinct proliferative and survival advantages for a cancer cell. For example, activating mutations in PI3K3C (a subunit of PI3K), and loss-of-function mutations in PTEN both upregulate Akt activity and are some of the most commonly mutated genes in cancer.

Growth factor signaling through MAPK Erk1/2 can inhibit apoptosis through phosphorylation of Bim at multiple sites, including Ser55, Ser65, and Ser73, which promotes its proteasomal degradation. In addition, signaling through Erk1/2 and PKC to p90RSK leads to phosphorylation and inhibition of Bad and increased expression of the anti-apoptotic factors Bcl-2 and Bcl-xL via CREB. Apoptosis can also be prevented through TNFR-mediated signaling to NF-κB, resulting in induction of XIAP and FLIP.

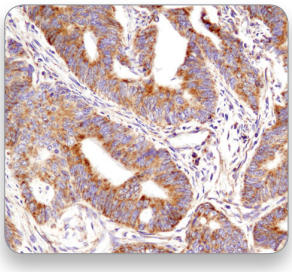
Select Reviews

Delbridge, AR, Valente, LJ, and Strasser, A. (2012) *Cold Spring Harb. Perspect. Biol.* 4 (11). | Brumatti, G, Salmanidis, M, and Ekert, PG. (2010) *Cell. Mol. Life Sci.* 67, 1619–1630. | Fuchs, Y and Steller, H. (2011) *Cell* 147, 742–758. | Shortt, J and Johnstone, RW. (2012) *Cold Spring Harb. Perspect. Biol.* 4 (12) | Hanahan, D and Weinberg, RA. (2011) *Cell* 144, 646–674.

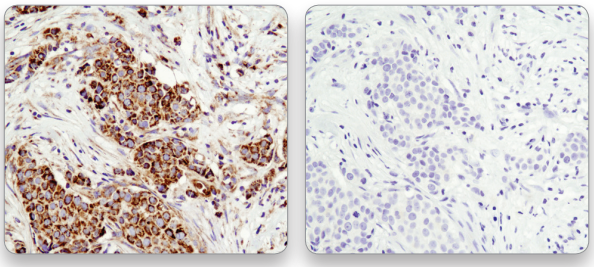
Antibodies to key targets as cellular readouts



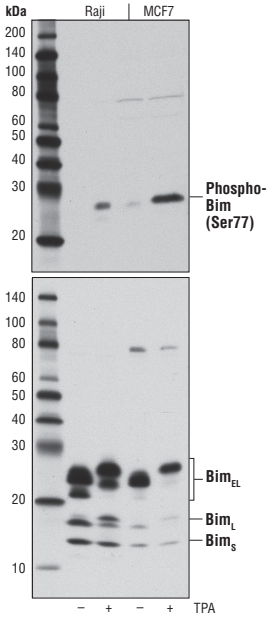
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060: IHC analysis of PTEN heterozygous mutant mouse endometrium using #4060. (Tissue section courtesy of Dr. Sabina Signoretti, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.)



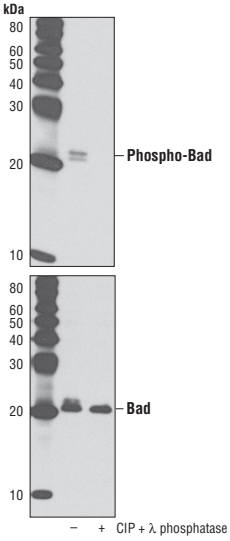
Bak (D4E4) Rabbit mAb #12105: IHC analysis of paraffin-embedded human colon carcinoma using #12105.



Cytochrome c (D18C7) Rabbit mAb #11940: IHC analysis of paraffin-embedded human breast carcinoma using Cytochrome c (D18C7) Rabbit mAb in the presence of control peptide (left) or antigen-specifc blocking peptide (right).

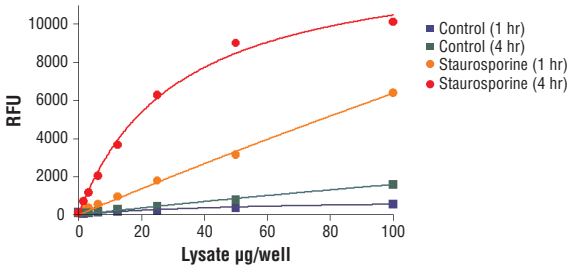


Phospho-Bim (Ser77) (D4H12) Rabbit mAb #12433: WB analysis of extracts from Raji and MCF7 cells, untreated (-) or treated with TPA #4174 (200 nM, 30 min; +), using #12433 (upper) or Bim (C34C5) Rabbit mAb #2933 (lower).



Phospho-Bad (Ser136) (D25H8) Rabbit mAb #4366: WB analysis of extracts from COS-7 cells, treated with Human Epidermal Growth Factor #8916 (100 ng/ml, 30 min) in the presence or absence of calf intestinal phosphatase (CIP) plus lambda-phosphatase, using #4366 (upper) or Bad (D24A9) Rabbit mAb #9239 (lower).

Assay kits to measure apoptosis



Caspase-3 Activity Assay Kit #5723: NIH/3T3 cells were treated with Staurosporine #9953 (5 µM, 5 hr) and then lysed in PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 (supplied with kit). Various amounts of cell lysate were added to assay plates containing the substrate solution, and plates were incubated at 37°C in the dark. Relative fluorescent units (RFUs) were acquired at 1 and 4 hr.

Antibody sampler kits offer a convenient and economical means for the simultaneous analysis of multiple nodes within a pathway of interest.

#8678 Kit Includes:
E-Cadherin (24E10) Rabbit mAb #3195, p53 (7F5) Rabbit mAb #2527, Stathmin Antibody #3352, BRCA1 Antibody #9010, Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060, PTEN (138G6) Rabbit mAb #9559, Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb #5587, HER2/ErbB2 (D8F12) XP® Rabbit mAb #4290, Anti-rabbit IgG, HRP-linked Antibody #7074

For a complete listing of our Antibody Sampler Kits:

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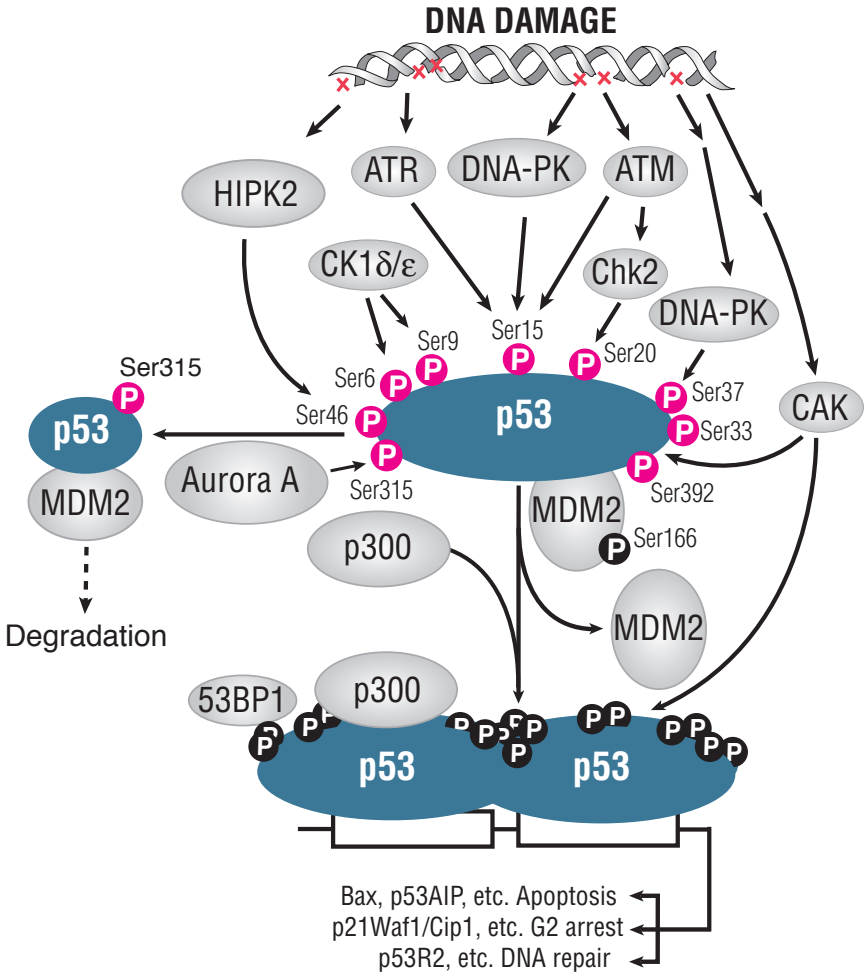
p53 Signaling
and the DNA Damage Response

Rigorously tested antibodies and controls to move your research forward

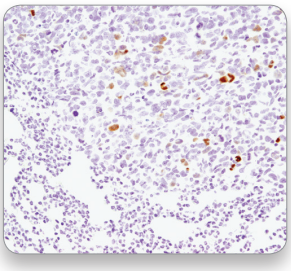


Cytotoxic stresses, such as ionizing radiation and hypoxia can cause single- or double-strand breaks to cellular DNA. The DNA damage response (DDR) is an important cellular mechanism that prevents the cell from replicating before the damage is repaired or the cell containing damaged DNA is eliminated. The p53 tumor suppressor protein is a transcriptional regulator that plays a major role in DDR. DNA damage induces phosphorylation of p53 at multiple sites by a number of upstream kinases including ATM, ATR, DNA-PK, and Chk2. Phosphorylation of p53 at Ser15 and Ser20 leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2. MDM2 inhibits p53 accumulation by targeting it for ubiquitination and proteasomal degradation. Activated p53 binds to target genes to induce a number of effector pathways including cell cycle arrest, DNA repair, senescence, or apoptosis. Studies have shown p53 to promote apoptosis through upregulation of the pro-apoptotic factors Puma and Noxa. Loss-of-function mutations in p53 or other elements of the DDR can result in cancer. In fact, up to 50% of human cancers may contain mutations in p53 or elements of the p53 signaling pathway.

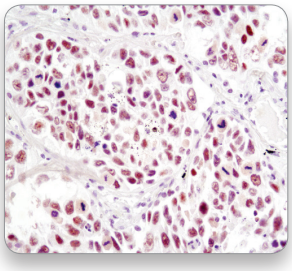
Select Reviews

Wade, M, Li, YC, and Wahl, GM. (2013) *Nat. Rev. Cancer* 13, 83-96.v | Muller, PA and Vousden, KH. (2013) *Nat. Cell Biol.* 15, 2–8. Sperka, T, Wang, J, and Rudolph, KL. (2012) *Nat. Rev. Mol. Cell Biol.* 13, 579–590. | Delbridge, AR, Valente, LJ, and Strasser, A. (2012) *Cold Spring Harb. Perspect. Biol.* 4 (11).

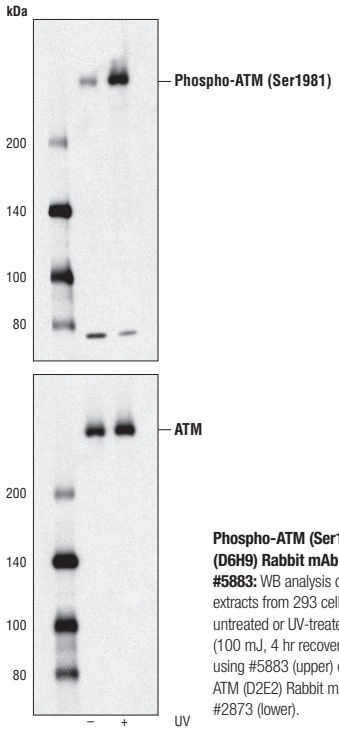
Antibodies to key targets as cellular readouts for the DNA Damage Response



Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Mouse Specific) #12571: IHC analysis of paraffin-embedded 4T1 metastatic tumors in mouse lung using #12571.

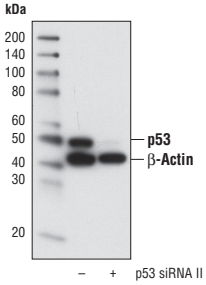


Chk2 (D9C6) XP® Rabbit mAb #6334: IHC analysis of paraffin-embedded human lung carcinoma using #6334.



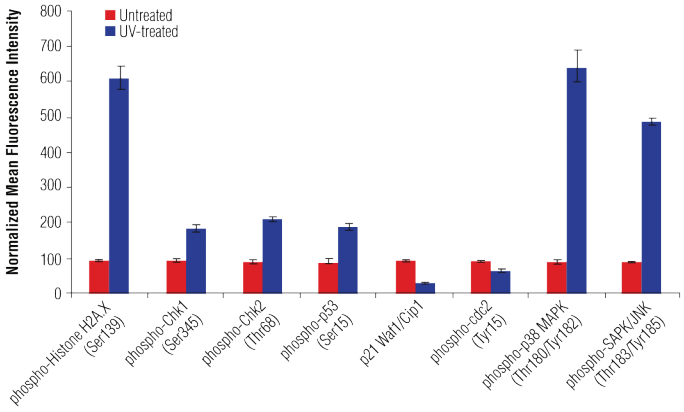
Phospho-ATM (Ser1981) (D6H9) Rabbit mAb #5883: WB analysis of extracts from 293 cells, untreated or UV-treated (100 mJ, 4 hr recovery), using #5883 (upper) or ATM (D2E2) Rabbit mAb #2873 (lower).

SignalSilence® siRNA for knockdown studies



SignalSilence® p53 siRNA II #6562: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 (-) #6562 (+), using p53 (1C12) Mouse mAb #2524 and β-Actin (13E5) Rabbit mAb #4970. p53 (1C12) Mouse mAb confirms silencing of p53 expression and β-Actin (13E5) Rabbit mAb is used to control for loading and specificity of p53 siRNA.

PathScan® Multi-Target Kits for high content analysis



PathScan® Multi-Target HCA DNA Damage Kit #7101: HepG2 cells were left untreated (blue) or subjected to a 100 mJ UV treatment followed by a 1 hr recovery period (red). Mean fluorescence intensity was measured for antibodies in #7101. Data were generated on the Acumen eX3® HCS platform.

Disease Connection

Apoptosis is a tightly controlled process necessary for normal growth and development in multicellular organisms, playing an important role in tissue remodeling, tissue homeostasis, and wound repair. However, loss of properly regulated apoptosis can result in a host of disease states such as autoimmune diseases, neurodegenerative disorders, and cancer.

In the immune system, apoptosis plays an important role in immune tolerance and clearing T lymphocytes that recognize self-antigens. Mutations that interfere in this process can result in autoimmune disease. For example, studies have shown that loss-of-function mutations in the death receptor Fas have been linked to autoimmune lymphoproliferative syndrome (ALPS).

Faulty apoptotic signaling can also lead to neurodegenerative disorders. In Parkinson's disease, premature apoptosis in dopaminergic neurons can be caused by loss-of-function mutations in PINK1, a protein that normally provides protection against cytochrome c release, as well as through upregulation of extrinsic pathway death receptors. In both cases, aberrant apoptotic signaling ultimately leads to neuronal cell death and the patient's eventual cognitive decline.

The misregulation of apoptosis can be most acutely associated with cancer. Suppression of apoptosis is one of the key acquired capabilities necessary for malignant transformation to occur. Oncogenic driver mutations found throughout the intrinsic and extrinsic pathways promote cell survival and are commonly upregulated in various forms of cancer. Although the identification of driver mutations has lead to new therapeutic opportunities, overcoming mechanisms of resistance remains a continual challenge in the field of cancer biology.

Select Reviews

Renault, TT and Chipuk, JE. (2013) *Ann. NY Acad. Sci.*1285, 59-79. | Suzanne, M and Steller, H. (2013) *Cell Death Differ.* 20, 669–675. | Roulston, A, Muller, WJ, and Shore, GC. (2013) *Sci. Signal.* 6, pe12. | Favaloro, B, Allocati, N, Graziano V, et al. (2012) *Aging* 4, 330–349. | Shortt, J and Johnstone, RW. (2012) *Cold Spring Harb. Perspect. Biol.* 4(12).

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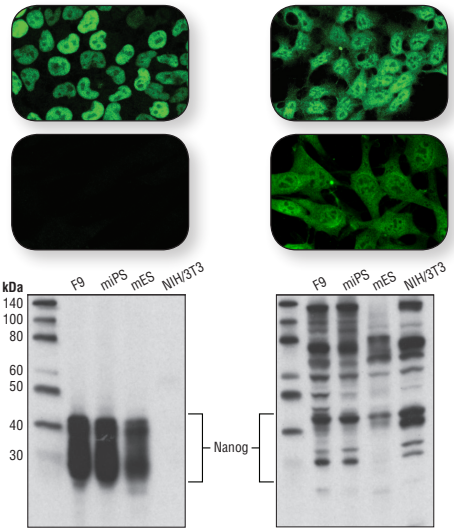
WB and IF analysis show that the other company's antibody lacks specificity

Nanog (D2A3) XP® Rabbit mAb (Mouse Specific) #8822 | **Another Company's Rabbit Polyclonal Antibody**



Seemingly comparable IF staining intensity for Nanog in F9 cells.

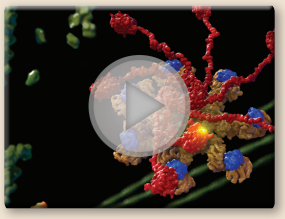
Non-specific IF staining in Nanog-null NIH/3T3 cells, using the antibody from the other company.

In WB, The antibody from the other company recognizes multiple non-specific bands and demonstrates weak reactivity with correct bands.

Nanog (D2A3) XP® Rabbit mAb (Mouse Specific) #8822: IF analysis of F9 (Nanog-positive) or NIH/3T3 (Nanog-null) cells using #8822 or another company's antibody (upper panel). WB analysis of various cell lines using #8822 or another company's antibody (lower panels).

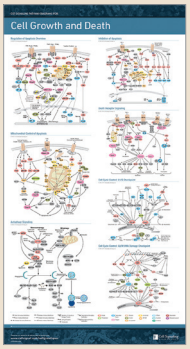
Apoptosome Formation and Activation Video

Visit **www.cellsignal.com/apoptosome** to view this short 3D rendered animation illustrating caspase-mediated apoptosome formation and activation from release of cytochrome c by mitochondria to prototeolytic degradation of cellular components such as actin filaments.



Apoptosis Signaling Pathways Poster

To request your copy of our Apoptosis Signaling Pathways poster, visit our resources page at: **www.cellsignal.com/apoptosis.**



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CST Product Scientists: Troy, PhD (left) has been with CST since 2010 and **Christina** (right) has been with CST since 2007.

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