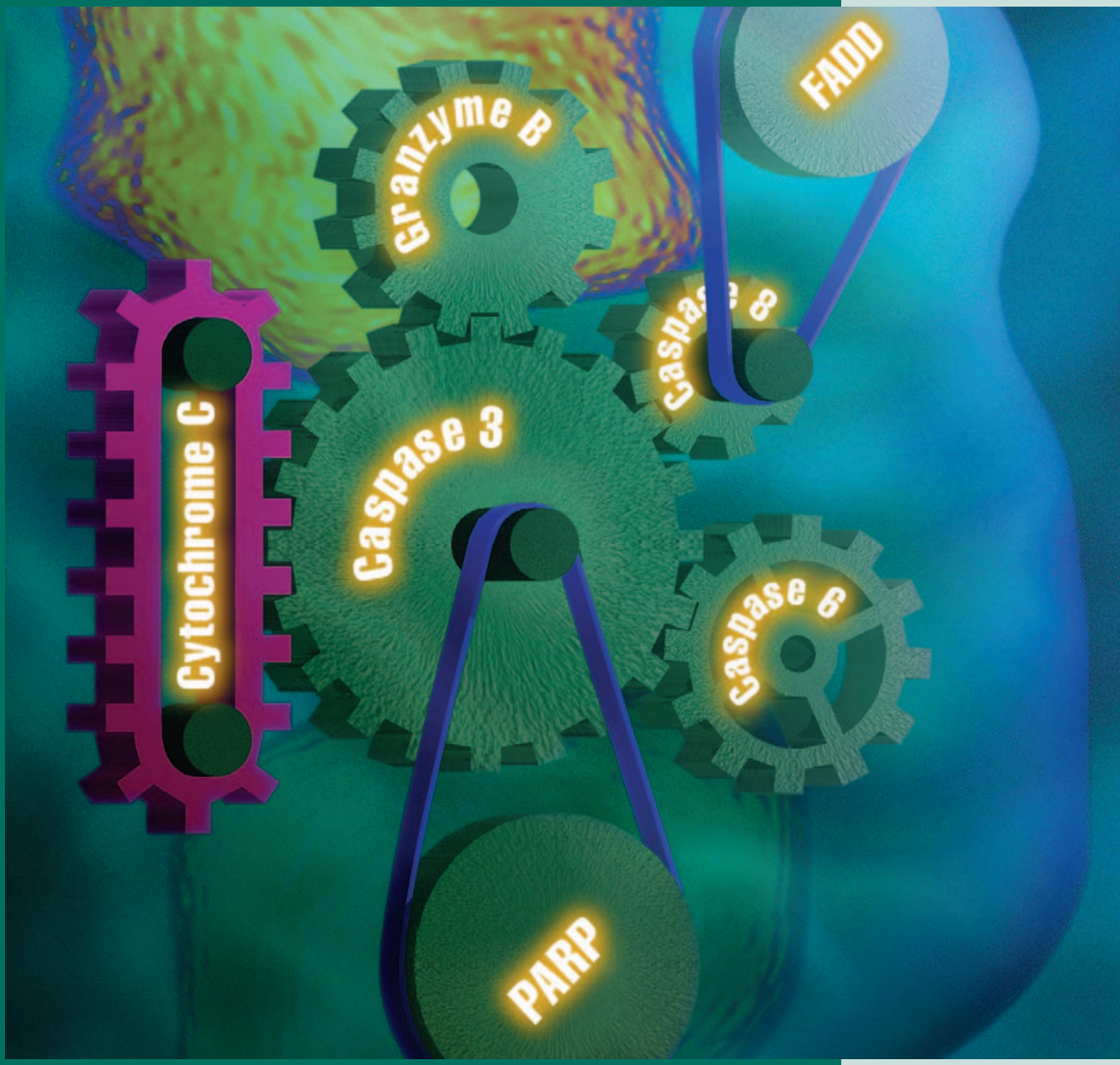
Apoptosis

Applied Reagents and Technologies



PharMingen
A Becton Dickinson Company

Featuring:

- New Antibody Specificities to:

  Caspase 6
  Caspase 7
  Caspase 9
  Caspase 10
  Apaf-1
  I-FLICE
  Daxx
  DFF
  DR3
  DR4

- Active Caspases 3, 6, 7, 8

- Annexin V-FITC Apoptosis Detection Kits

Coming Soon:

- Antibodies Specific for Active Caspase 3

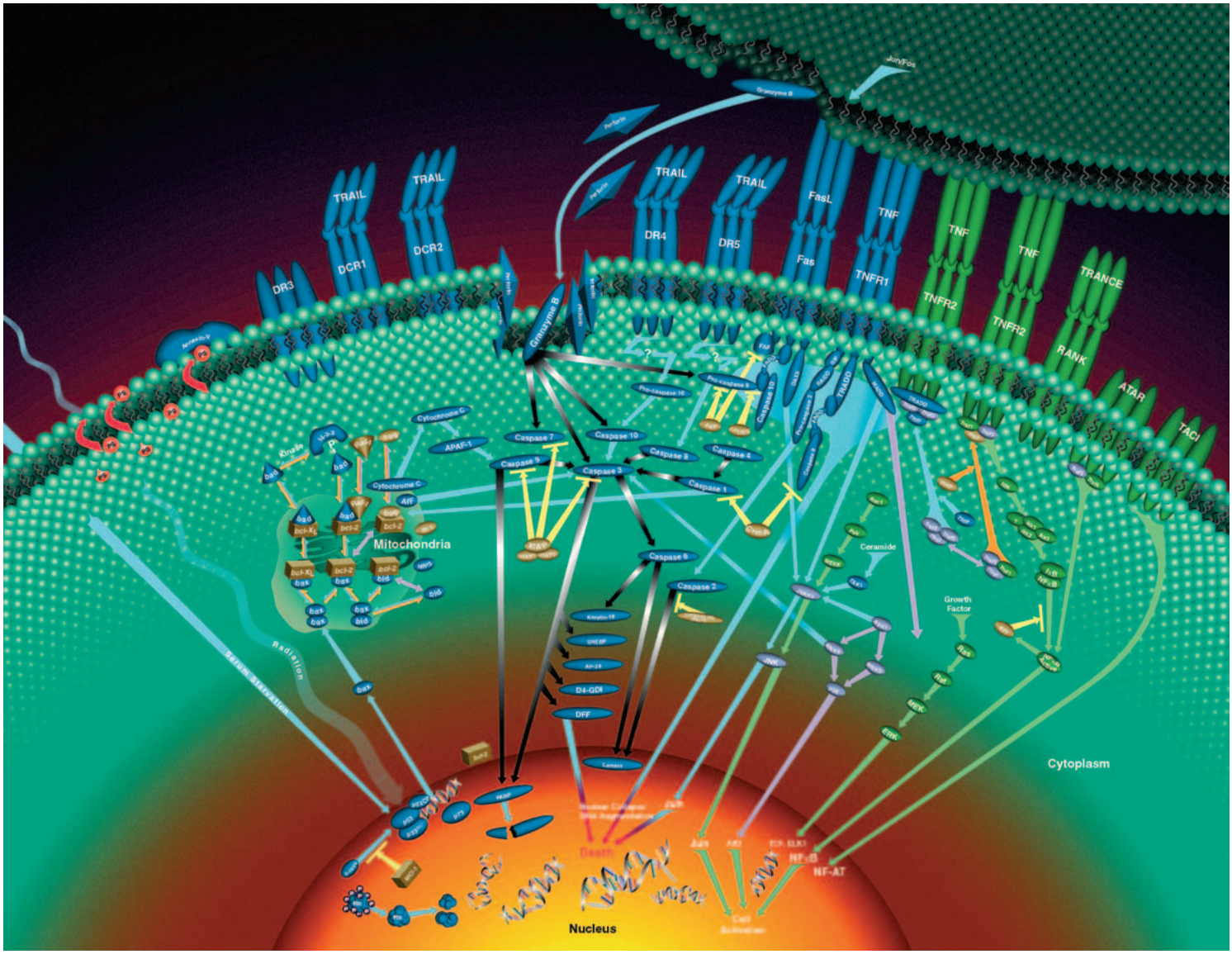
Table of Contents

APC= Allophycocyanin
Apps.= Applications
ASC= Ascites
E=ELISA
EBS= Epitope Blocking Studies
ENZ=Enzyme
F= FITC
FA=Functional Assays
FC= Flow Cytometry
F Set= FITC Set
Hu= Human
IF=Immunofluorescence
IHCF= Immunohistochemistry (Frozen)
IHCP= Immunohistochemistry (Paraffin)
IP= Immunoprecipitation
IVK=*In Vitro* Kinase Assay
IVT=*In Vitro* Transcription
Ms= Mouse
NL=NA/LE™=No Azide/Low Endotoxin®
PEP=Peptide
PU= Purified
R= Rat
RPA= Ribonuclease Protection Assay
RXNS= Reactions
SE= Antiserum
SF=Spectrofluorometry
WB= Western Blot

Introduction

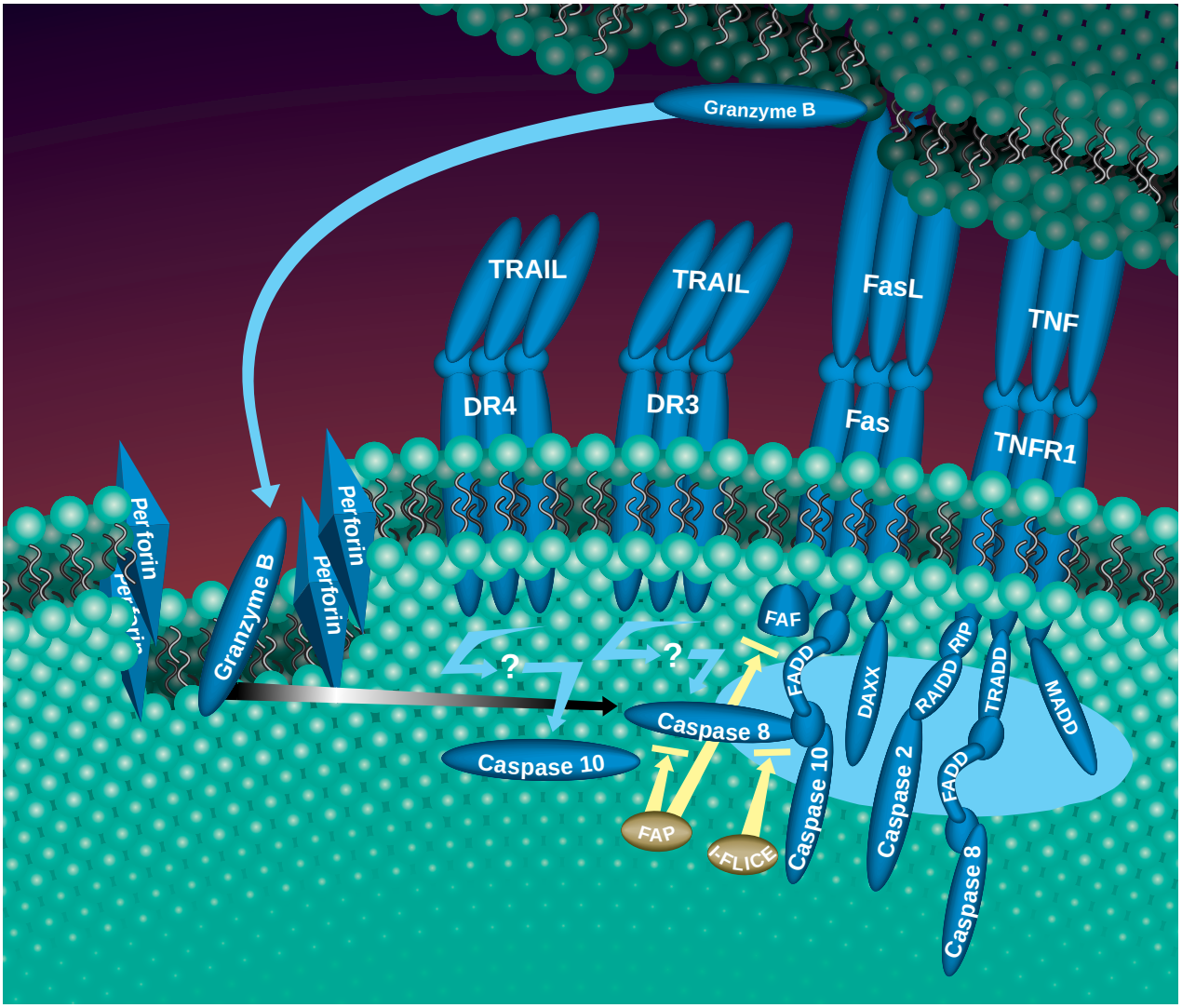
Programmed cell death is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. The term apoptosis, from the Greek word for "falling off" of leaves from a tree, is used to describe a process in which a cell actively participates in its own destructive processes. The apoptotic program is characterized by certain morphological features. These include changes in the plasma membrane such as loss of membrane asymmetry and attachment, a condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. In the final stages, the dying cells become fragmented into "apoptotic bodies" which are rapidly eliminated by phagocytic cells without eliciting significant inflammatory damage to surrounding cells. Inappropriate induction of apoptosis has broad ranging pathologic implications and has been associated with many autoimmune disorders.

The number of techniques designed to identify, quantitate, and characterize apoptosis is escalating as we learn more about the complex mechanisms which underlie this process. PharMingen offers a variety of reagents and detection systems for multi-parameter apoptosis research. Our product line includes antibodies to mouse, rat and human proteins including: Fas and Fas Ligand, TRAIL and DR3/DR4, Bcl-2 family members, caspases and other signaling molecules. For detection of apoptotic cells by flow cytometry, we now offer the Caspase-3 Assay Kit, APO-BRDU™ and APO-DIRECT™ Kits and Annexin V reagents. For mRNA analysis, the RiboQuant® RNase Protection Assay system features mouse, rat and human multi-probe template sets for apoptosis-related genes.

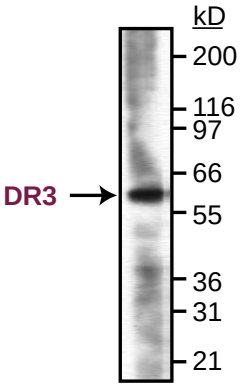


Death Receptors and Ligands

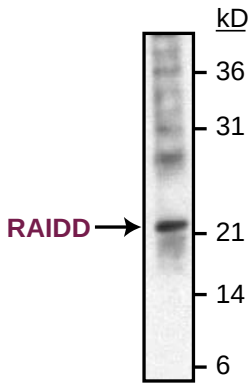
Fas Ligand (FasL) and TRAIL (Apo-2L) are members of the TNF superfamily of cytokines which are pleiotropic mediators of host defense and immune responses. Binding of FasL to its receptor, Fas, or of TRAIL to its receptors, initiates signal transduction pathways which result in the induction of apoptosis. The Fas/FasL system is involved in T cell development, clonal deletion of self-reactive T cells and other aspects of negative selection. TRAIL-mediated pathways involve activation of the transcription factor, NF-κB, as well as activation of several members of the caspase family of cysteine proteases. Several TRAIL receptors have been identified, which either activate TRAIL signal pathways, e.g., DR3, DR4 and DR5 or may act as "decoy" receptors, so-called DcRs, e.g., DcR1, DcR2.



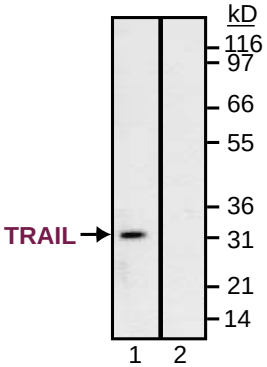
Orders 877.232.8995 • Phone 858.812.8800
World Wide Web http://www.bdbiosciences.com



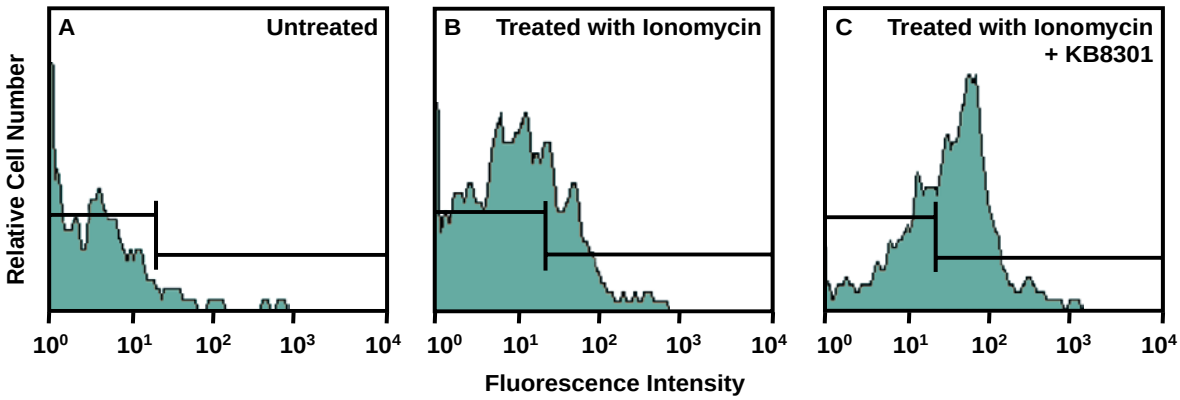
Western blot analysis of DR3, a receptor for the cytotoxic ligand TRAIL, in Jurkat T cell lysate. Affinity purified, rabbit anti-human DR3 (Cat. No. 67061N) identifies DR3 as an ~ 59 kD band.



Western blot analysis of RAIDD, also known as CRADD, in HeLa cervical carcinoma cell lysate. Affinity purified, rabbit anti-human RAIDD (Cat. No. 67081N) identifies RAIDD as an ~ 22 kD band.



Western blot analysis of TRAIL in WI-38 human diploid fibroblasts. Lane 1, clone B35-1, Cat. No. 66251A. Lane 2, a mouse IgG_{2a} isotype control. The B35-1 antibody identifies TRAIL as an ~32 kD band.



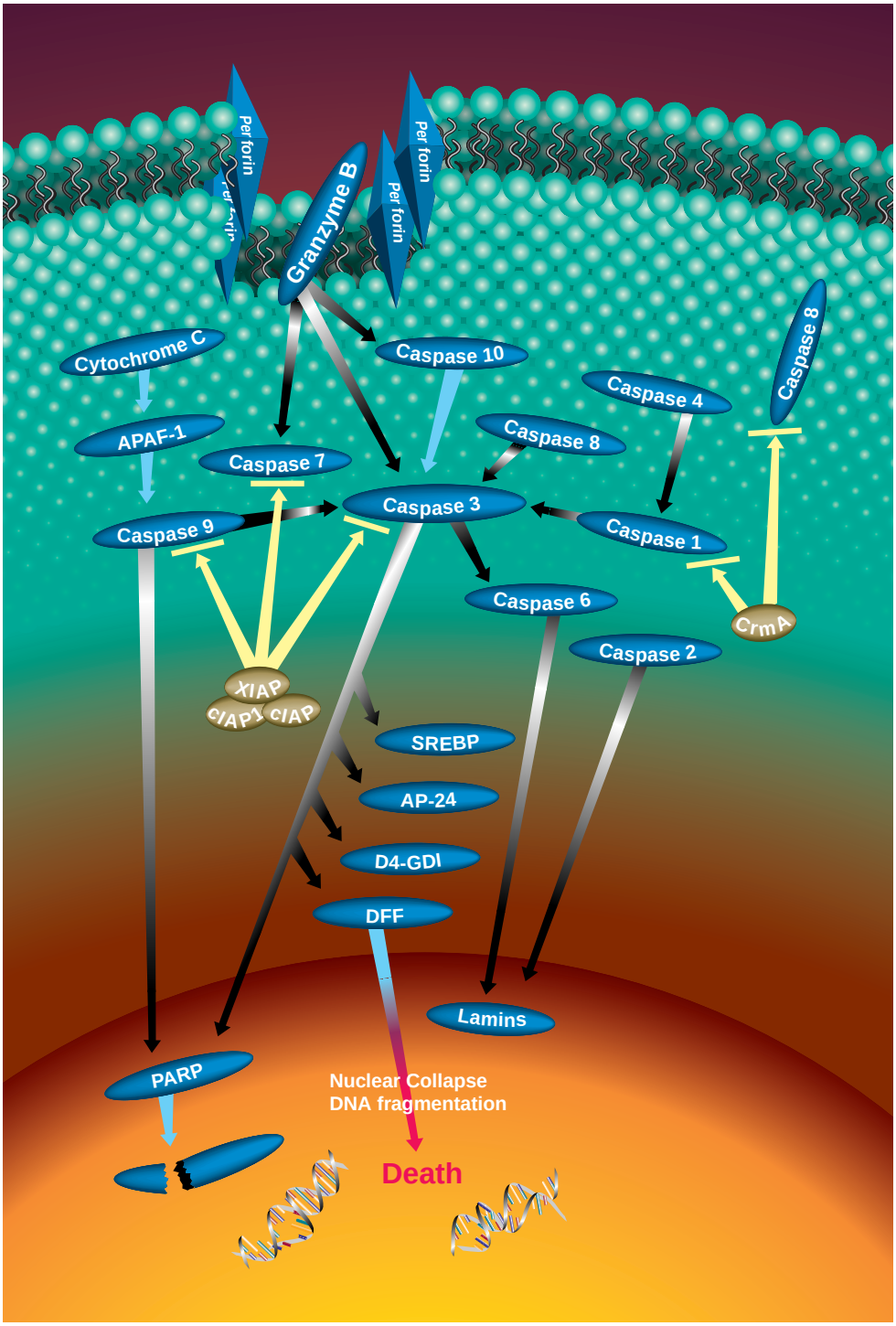
Flow cytometric analysis of Fas Ligand (FasL) in peripheral blood mononuclear cells (PBMCs). PBMCs were cultured for 3 hr in medium alone (A), Ionomycin (B), or Ionomycin and a matrix metalloproteinase inhibitor, KB8301 (Cat. No. 66131D). The cells were then stained for flow cytometry with purified FasL antibody, clone NOK-1 (Cat. No. 65321A), followed by goat anti-mouse IgG-biotin. The cells were then incubated with normal mouse serum before adding anti-CD16-FITC (Cat. No. 30624X) as a useful gate indicator and Streptavidin-PE (Cat. No. 13025D). Cells were analyzed on a FACScan™.

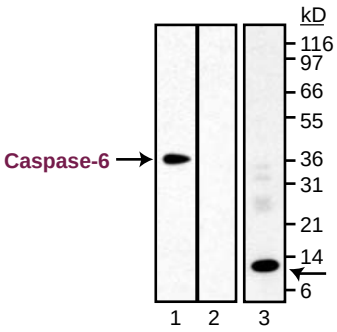
Description	Clone(s)	Specificity	Apps.	Format	Size	Cat. No.
Daxx	Polyclonal	Hu	WB	PU	50 µg	67051N
DR3	Polyclonal	Hu	WB	PU	50 µg	67061N
DR4	Polyclonal	Hu	WB	PU	50 µg	66901N
DR4	Polyclonal	Hu	WB	PU	50 µg	66891N
FADD	A66-2	Hu	IP, WB	PU	0.1 mg	65751A
FAF-1	B57-1	Hu	IP, WB	PU	0.1 mg	66411A
Fas	DX2	Hu	FA, FC	NL	0.5 mg	33450D
Fas	G254-274	Hu	WB	PU	0.1 mg	65311A
Fas	Jo2	Ms	IP, FC, FA	NL	0.5 mg	15400D
Fas Ligand	NOK-1	Hu	IP, FC, FA	NL	0.25 mg	65320C
Fas Ligand	NOK-2	Hu	IP, FA	NL	0.25 mg	65330C
Fas Ligand	G247-4	Hu	IP, WB, FC	PU	0.1 mg	65431A
Fas Ligand	Kay-10	Ms	FC, FA	NL	0.5 mg	09930D
			FC	PU	0.5 mg	09931D
			FC	PE	0.2 mg	09935B
Fas Ligand	MFL3	Ms	FC	PU	0.5 mg	28101D
Mcl-1	Polyclonal	Hu	IP, WB, IHCF, IHCP	SE	0.1 ml	13656E
Perforin	δG9	Hu	WB	PU	0.1 mg	65991A
Perforin Antibody Reagent Set	δG9,27-35	Hu	FC	PE Set	100 tests	6599KK
RAIDD	Polyclonal	Hu	WB	PU	50 µg	67081N
RIP	G322-2	Hu	IP, WB	PU	0.1 mg	65591A
TRADD	B36-2	Hu	WB	PU	0.1 mg	66431A
TNF-α	MAb11	Hu	FC	PU	0.1 mg	18641A
			FC	F	0.1 mg	18644A
			FC	PE	0.1 mg	18645A
			FC	APC	0.1 mg	18649A
TNF-α	MP6-XT22	Ms	FC	PU	0.1 mg	18131A
			FC	F	0.1 mg	18134A
			FC	PE	0.1 mg	18135A
			FC	APC	0.1 mg	18139A
TNF-β	359-81-11	Hu	FC, FA	NL	0.5 mg	18910D
			FC	PU	0.1 mg	18911A
			FC	PE	0.1 mg	18915A
TRAF3	Polyclonal	Hu	WB, IHCP	SE	0.1 ml	66536E
TRAF3	B1-6	Hu	WB	PU	0.1 mg	66191A
TRAF4	Polyclonal	Hu	IHCP	SE	0.1 ml	66546E
TRAIL	B35-1	Hu	WB	PU	0.1 mg	66251A
Related Reagents						
Human Fas: FC Chimeric Fusion Protein	Recombinant		IP, FC, FA	PU	100 µg	67231A
TNF-α	Recombinant	Hu	FA	PU	10 µg	19761T
TNF-α	Recombinant	Ms	FA	PU	10 µg	19321T
TNF-α	Recombinant	Hu	FA	PU	10 µg	19771T
OptEIA™ Soluble Fas		Hu	E	Set	20 x 96 tests	2649KI
OptEIA™ TNF-α		Hu	E	Set	20 x 96 tests	2637KI
OptEIA™ TNF-α		Ms	E	Set	20 x 96 tests	2673KI

Caspases

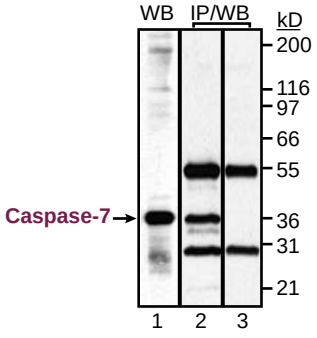
Despite the diversity of signals which can induce cell death, in their execution these pathways can share several features. One mechanism which is consistently implicated in apoptosis is the activation of a series of cytosolic proteases, the caspases. The caspase family of cysteine proteases was discovered following a search of human cDNA libraries for sequences homologous to *ced-3*, a cell death gene described in the nematode worm, *C. elegans*. The first mammalian homologue of *ced-3* to be identified was ICE (interleukin-1β converting enzyme). Subsequently, multiple mammalian *ced-3* homologues were discovered and each given a variety of names: caspase-1 (ICE), caspase-2 (ICH-1), caspase-3 (CPP32, Yama, apopain), caspase-4 (TX, ICH-2, ICErel-II), caspase-5 (ICErel-III), caspase-6 (Mch2), caspase-7 (Mch3, ICE-LAP3, CMH-1), caspase-8 (MACH, FLICE, Mch5), caspase-9 (ICE-LAP6, Mch6) and caspase-10 (Mch4, FLICE2). To achieve consistency the term "caspase" was adopted as a root name for all family members. The name reflects the catalytic properties of these enzymes, the "c" denotes their cysteine protease mechanism and "aspase" refers to their ability to cleave after aspartic acid residues. Caspases 1 through 10 have been grouped according to sequence homology as being either ICE-like (caspases 1, 4 and 5) or ced-3 like (caspases 3, 6, 7, 9 and 10). Caspases are synthesized as inactive "proenzymes" that are processed by proteolytic cleavage to form an active enzyme.

Caspase-3 is a key protease that becomes activated during the early stages of apoptosis. Active caspase-3, found in cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kD subunits which are derived from the 32 kD proenzyme. In its active form, caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm (e.g., D4-GDI) and nucleus (e.g., PARP).

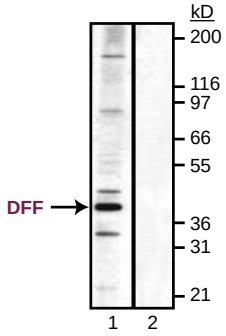




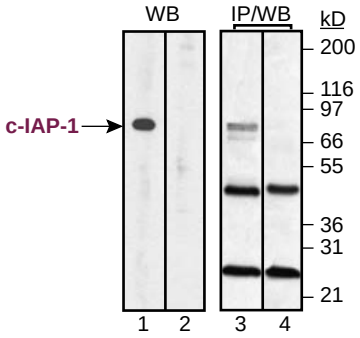
Western blot analysis of caspase-6. Lanes 1 and 2, Daudi B cell lysate was probed with anti-human caspase-6 (clone B93-4; Cat. No. 68041A) or with a mouse IgG_1 isotype control. Clone B93-4 identifies full length caspase-6 as an ~ 34 kD band (lane 1). Lane 3, Purified, Active Caspase-6 (Cat. No. 66291T) which exists as a proteolytically cleaved dimer of 18 kD and 11 kD subunits, was probed with clone B93-4. The antibody identifies the 11 kD subunit of the active caspase-6.



Immunoprecipitation/Western blot analysis of human caspase-7 in 293 embryonic kidney cells. Lane 1: 293 cell lysate was probed directly with B94-1 (Cat. No. 66871A) which identifies caspase-7 at ~35 kD. Lanes 2 and 3: 293 cell lysate was immunoprecipitated with clone B94-1 (lane 2) or with an isotype control (lane 3) and detected by western blot analysis with B94-1. The bands above and below the specific band at ~35 kD represent the heavy and light chains of IgG used for immunoprecipitation.



Western blot analysis of DFF in HeLa cervical carcinoma cell lysate. Affinity purified, rabbit anti-human DFF (Cat. No. 66911N) identifies full length (uncleaved) DFF as an ~45 kD band.



Immunoprecipitation/Western blot analysis of c-IAP-1. Lanes 1 and 2: Jurkat T cell lysate was probed with clone B75-1 (Cat. No. 66791A) (lane 1) or with an isotype control (lane 2). B75-1 identifies c-IAP-1 as an ~72kD band. Lanes 3 and 4: 293 cell lysate was immunoprecipitated with clone B75-1 (lane 3) or with an isotype control (lane 4) and detected by western blot analysis with B75-1.The bands below ~72kD in lanes 3 and 4 represent the heavy and light chains of IgG used for immunoprecipitation.

Description	Clone(s)	Specificity	Apps.	Format	Size	Cat. No.
APAF-1	Polyclonal	Hu	IP	SE	0.1 ml	68066E
APAF-1	Polyclonal	Hu	WB	SE	0.1 ml	68076E
Caspase-1	B24-1	Hu, Ms	WB	PU	0.1 mg	66441A
Caspase-2	G310-1248	Hu, Ms	WB	PU	0.1 mg	13951A
Caspase-3	Polyclonal	Hu, Ms	WB, IHCP	SE	0.1 ml	65906E
Caspase-4	B25-1	Hu, Ms	WB	PU	0.1 mg	66171A
Caspase-6	B93-4	Hu	WB	PU	0.1 mg	68041A
Caspase-7	B94-1	Hu	IP, WB	PU	0.1 mg	66871A
Caspase-8	B9-2	Hu	WB	PU	0.1 mg	66231A
Caspase-9	B40	Hu	WB	PU	0.1 mg	66571A
Caspase-9	Polyclonal	Hu	WB	SE	0.1 ml	68086E
Caspase-10	Polyclonal	Hu	WB	PU	50 µg	67041N
c-IAP	B75-1	Hu	IP, WB	PU	0.1 mg	66791A
Crm A	A71-1	Viral Protein	WB	PU	0.1 mg	65921A
Cytochrome c	6H2.B4	Hu, Ms, R	IP	PU	0.1 mg	65971A
Cytochrome c	7H8.2C12	Hu, Ms, R	WB	PU	0.1 mg	65981A
D4-GDI	Polyclonal	Hu	WB	SE	0.1 ml	66456E
Rho-GDI/D4-GDI	Polyclonal	Hu	WB	SE	0.1 ml	66586E
DFF	Polyclonal	Hu	IP, WB	PU	50 µg	66911N
DFF	Polyclonal	Hu	WB	PU	50 µg	66921N
I-FLICE	Polyclonal	Hu	WB	PU	50 µg	67071N
PARP	4C10-5	Hu	IP, WB, FC	PU	0.1 mg	66401A
PARP	7D3-6	Hu	IP, WB, FC	PU	0.1 mg	66391A
PARP	C2-10	Hu, Ms, R	WB	ASC	0.1 ml	65196E

Related Reagents

Caspase-3 Assay Kit		FC, SF	Kit	100 tests	6632KK
Active Caspase-3 Set		SF	Set	20 tests	6628KK
Active Caspase-6 Set		SF	Set	20 tests	6629KK
Active Caspase-7 Set		SF	Set	20 tests	6630KK
Active Caspase-8 Set		SF	Set	20 tests	6631KK
Ac-YVAD-AMC, Caspase-1 Fluorogenic Substrate		SF	PEP	1 mg	66091U
Ac-YVAD-CHO, Caspase-1 Inhibitor		SF	PEP	1 mg	66591U
Ac-DEVD-AFC, Caspase-3 Fluorogenic Substrate		SF	PEP	1 mg	67201U
Ac-DEVD-AMC, Caspase-3 Fluorogenic Substrate		SF	PEP	1 mg	66081U
Ac-DEVD-CHO, Caspase-3 Inhibitor		SF	PEP	1 mg	66221U
Ac-VEID-AFC, Caspase-6 Fluorogenic Substrate		SF	PEP	1 mg	66941U
Ac-VEID-CHO, Caspase-6 Inhibitor		SF	PEP	1 mg	66951U
Ac-IETD-AFC, Caspase-8 Fluorogenic Substrate		SF	PEP	1 mg	66961U
Ac-IETD-CHO, Caspase-8 Inhibitor		SF	PEP	1 mg	66971U
Purified, Active Recombinant Caspase-3	Hu	SF, FA	ENZ	5 µg/10 µg	66281V/66281T
Purified, Active Recombinant Caspase-6	Hu	SF, FA	ENZ	5 µg/10 µg	66291V/66291T
Purified, Active Recombinant Caspase-7	Hu	SF, FA	ENZ	5 µg/10 µg	66301V/66301T
Purified, Active Recombinant Caspase-8	Hu	SF, FA	ENZ	5 µg/10 µg	66311V/66311T
Recombinant mutant Crm A Protein		WB, FA	PU	50 µg	66601N
Recombinant wildtype Crm A Protein		WB, FA	PU	50 µg	66611N

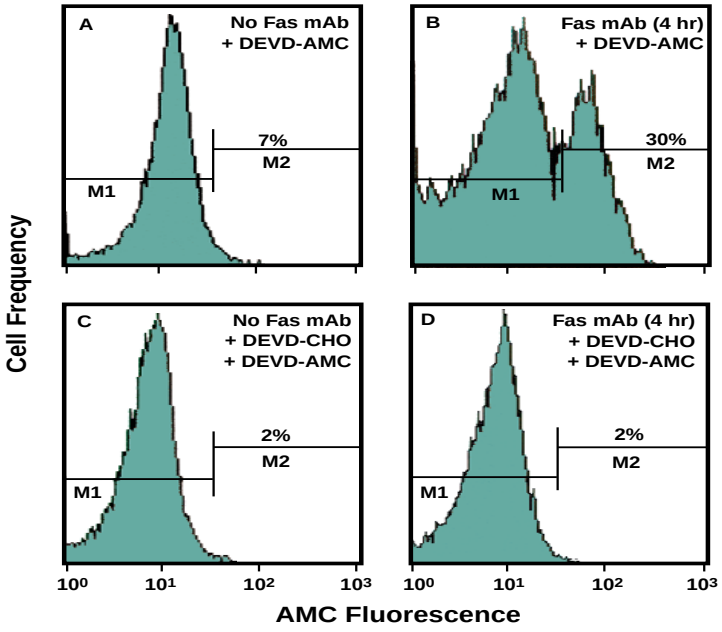
Caspases

Caspase-3 Assay Kit

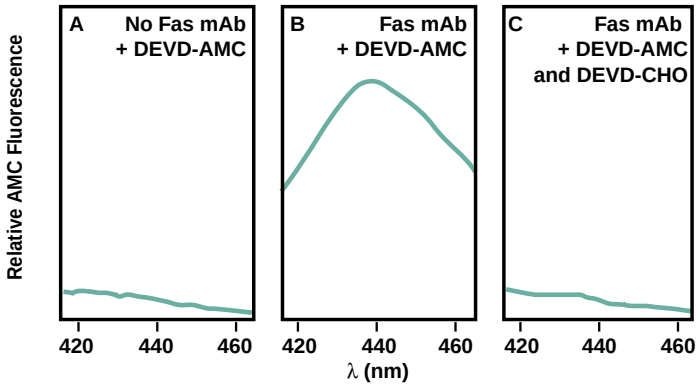
This kit is designed to measure caspase-3 activity, an early marker of cells undergoing apoptosis, and as such can be used to determine if certain apoptosis pathways involve activation of caspase-3. The kit contains a caspase-3 fluorogenic substrate, a caspase-3 aldehyde inhibitor and assay buffers.

Ac-DEVD-AMC is a synthetic tetrapeptide fluorogenic substrate that is used to identify and quantitate caspase-3 activity in apoptotic cells. Caspase-3 cleaves the tetrapeptide between D and AMC, releasing fluorescent AMC which can be measured by flow cytometry or spectrofluorometry. Ac-DEVD-CHO is a potent synthetic tetrapeptide inhibitor of caspase-3 activity that can be used to block the fluorogenic signal which results from caspase-3-mediated cleavage of Ac-DEVD-AMC. Thus, when apoptotic cells or cell lysates are incubated with both substrate and inhibitor, caspase-3 activity is blocked by Ac-DEVD-CHO, AMC is not released from Ac-DEVD-AMC and fluorescence is not detected.

Note: These methods require instrumentation which is capable of UV excitation, 380 nm, and detection of an emission wavelength range of 420-460 nm.



Flow cytometric analysis of Caspase-3 activity. Jurkat T cells were untreated (A, C) or treated with anti-human Fas antibody, clone DX2 (Cat. No. 33450D), and Protein G (B, D) for 4 hr to induce apoptosis. Cells were incubated with the Ac-DEVD-AMC, Caspase-3 substrate (A,B) or the substrate and the Ac-DEVD-CHO inhibitor (C, D) and analyzed by UV flow cytometry (FacSTAR™). A, C) Untreated cells did not emit fluorescence, indicating that the substrate was not cleaved and hence Caspase-3 activity was absent. B) The substrate was cleaved in about one third of the population treated with anti-Fas mAb, indicating the presence of Caspase-3 activity. D) Fluorescence was not emitted in anti-Fas mAb treated cells when both the inhibitor and substrate were added, indicating that Caspase-3 activity was blocked.



Spectrofluorometric analysis of Caspase-3 activity. Lysates were prepared from Daudi B cells that were untreated (A) or treated with anti-human Fas antibody, clone DX2 (Cat. No. 33450D), and Protein G (B,C) for 4 hr to induce apoptosis. Cells were incubated with the Ac-DEVD-AMC, Caspase-3 substrate (A, B) or the substrate and the Ac-DEVD-CHO inhibitor (C) and analyzed by spectrofluorometry. A) Lysates from untreated cells did not emit fluorescence, indicating that the substrate was not cleaved and hence Caspase-3 activity was absent. B) Lysates from cells treated with anti-Fas mAb cleaved the substrate, indicating the presence of Caspase-3 activity. C) Fluorescence was not emitted in lysates from cells treated with anti-Fas mAb when both the inhibitor and substrate were added, indicating that Caspase-3 activity was blocked.

Description	Size	Cat. No.
Caspase-3 Assay Kit	100 tests	6632KK

Kit Components

Ac-DEVD-AMC, Caspase-3	5 x 200 µg
Ac-DEVD-CHO, Caspase-3	5 x 200 µg
PBS Buffer, 10X Concentrate	50 ml
Cell Lysis Buffer, 1X Concentrate	50 ml
HEPES Buffer, 2X Concentrate	50 ml

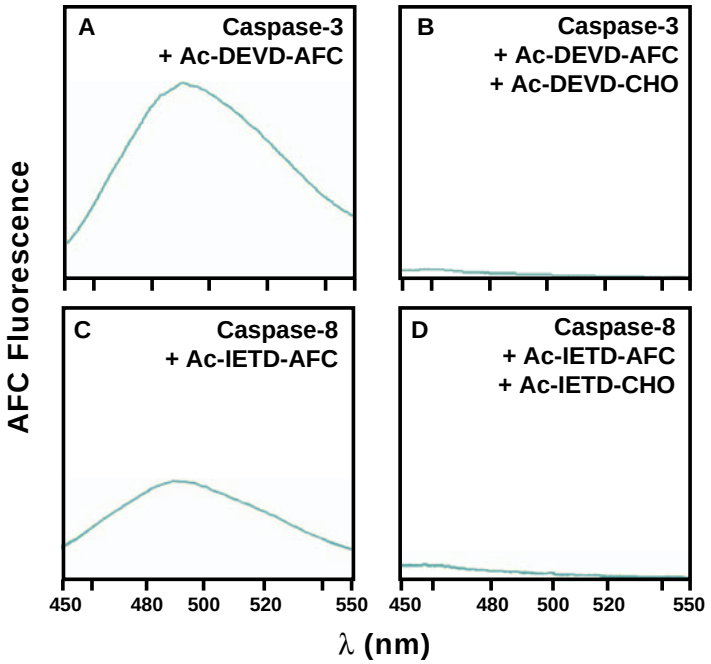
Caspases

Active Caspase Sets

Heterologous expression systems have been used to produce active, recombinant forms of human caspases, providing the investigator with an additional tool for characterization of apoptotic pathways. While active caspase enzymes may be incorporated into any number of experimental designs, each of PharMingen's Active Caspase Sets includes the active caspase of interest as well as a specific fluorogenic substrate and inhibitor which are useful controls to test the activity of the enzymes in your assays.

These reagents are suggested for use in experiments in which the activity of a specific caspase enzyme is desired. For example, one can determine whether a putative caspase substrate is cleaved by the active enzyme. However, these reagents are not designed to quantify the activity of endogenous caspases (*in vivo*). This is because the fluorogenic substrates provided with each set are not cell permeable and therefore cannot be cleaved by intracellular caspases for quantitative analysis. To identify and quantitate caspase-3 activity in apoptotic cells, please refer to our Caspase-3 Assay Kit described on page 6.

Note: These methods require instrumentation which is capable of UV excitation, 400 nm, and detection of an emission wavelength range of 480-520 nm.

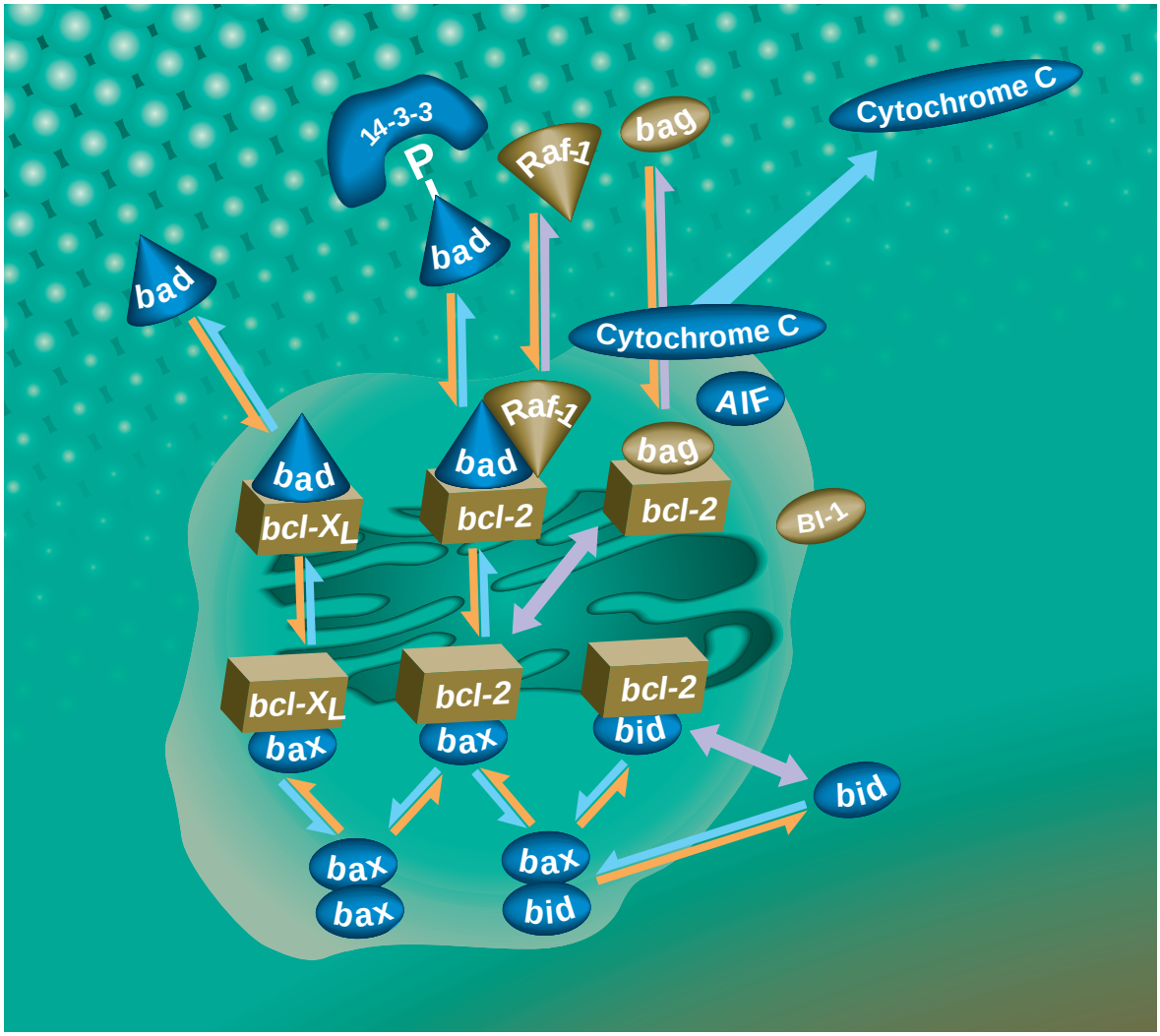


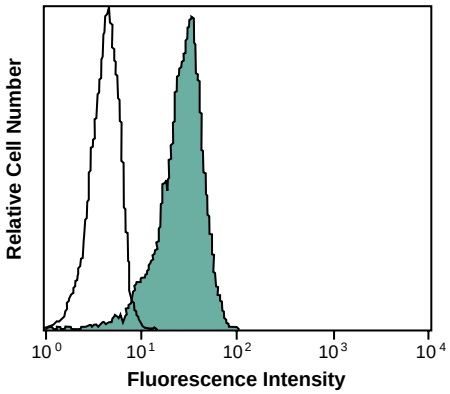
Activity of recombinant human caspase-3 and caspase-8. Ac-DEVD-AFC and Ac-IETD-AFC are synthetic tetrapeptide substrates that are cleaved by active human caspases. These substrates are cleaved between D and AFC, releasing the fluorogenic AFC, which is detected by spectrofluorometry. Caspase-3 cleaves Ac-DEVD-AFC, whereas caspase-8 cleaves Ac-IETD-AFC. When coupled to an aldehyde group (CHO), these tetrapeptides function as potent inhibitors of caspase activity and can be used to block caspase cleavage of Ac-DEVD-AFC or Ac-IETD-AFC. A) In the presence of caspase-3, fluorogenic AFC is released from Ac-DEVD-AFC, demonstrating the activity of caspase-3. B) In the presence of both caspase-3 and Ac-DEVD-CHO, fluorogenic AFC is not released, indicating that Ac-DEVD-AFC was not cleaved and that caspase-3 activity was blocked by Ac-DEVD-CHO. C) In the presence of caspase-8, fluorogenic AFC is released from Ac-IETD-AFC, demonstrating the activity of caspase-8. D) In the presence of both caspase-8 and Ac-IETD-CHO, fluorogenic AFC is not released, indicating that Ac-IETD-AFC was not cleaved and that caspase-8 activity was blocked by Ac-IETD-CHO.

Description	Size	Cat. No.
Active Caspase-3 Set	20 tests	6628KK
Kit Components		
Active Caspase-3	5 µg	
Ac-DEVD-AFC	200 µg	
Ac-DEVD-CHO	20 µg	
Active Caspase-6 Set	20 tests	6629KK
Kit Components		
Active Caspase-6	5 µg	
Ac-VEID-AFC	200 µg	
Ac-VEID-CHO	20 µg	
Active Caspase-7 Set	20 tests	6630KK
Kit Components		
Active Caspase-7	5 µg	
Ac-DEVD-AFC	200 µg	
Ac-DEVD-CHO	20 µg	
Active Caspase-8 Set	20 tests	6631KK
Kit Components		
Active Caspase-8	5 µg	
Ac-IETD-AFC	200 µg	
Ac-IETD-CHO	20 µg	

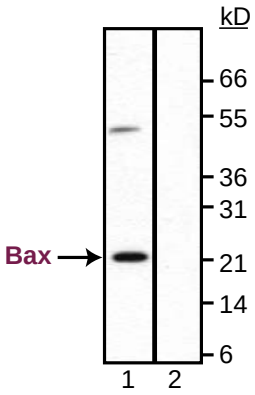
Bcl-2 Family Members

Members of the Bcl-2 family play an important role in regulating the response of cells to a wide variety of apoptotic signals. Bcl-2 is considered to be novel among protooncogenes because it blocks apoptosis in many cell types, thus providing selective survival advantage for cells and possibly contributing to tumorigenesis. Other members of this family which act as inhibitors of apoptosis include Bcl-X_L, Mcl-1 and A1. Several Bcl-2 family members also promote cell death, e.g., Bad, Bak, Bax and Bcl-X_S. It is thought that protein-protein interactions between Bcl-2 family members play an important role in their function. For example, Bax may form homodimers or heterodimers with either Bcl-2 and Bcl-X_L. Formation of Bax homodimers is thought to promote cell death, whereas Bax heterodimerization with either Bcl-2 or Bcl-X_L appears to block cell death. Bad, a pro-apoptotic Bcl-2 family member, heterodimerizes with Bcl-2 and Bcl-X_L, but not with other Bcl-2 family members. When Bad heterodimerizes with Bcl-X_L, it displaces Bax from Bax-Bcl-X_L heterodimers and promotes cell death. More recently, members of this family have been suggested to play a role in ion channel formation *in vitro*.

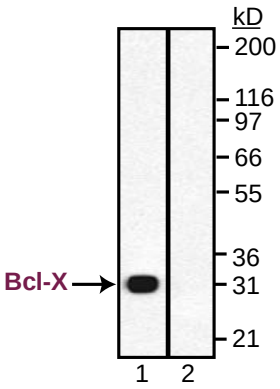




Flow cytometric analysis of Bcl-2 in human peripheral blood lymphocytes. Cells were stained with Bcl-2/100 Antibody Reagent Set (Cat. No. 6511KK), which includes anti-human Bcl-2-FITC (clone Bcl-2/100) and an IgG_1-FITC isotype control. Cells were then analyzed on a FACScan™.



Western blot analysis of Bax in Daudi B cell lysate. Lane 1, clone 6A7 (Cat. No. 66241A) detects Bax as an ~21 kD band. To demonstrate specificity, the binding of clone 6A7 to the Bax protein was blocked by preincubation with 6A7 specific blocking peptide which is part of the Bax (6A7) Blocking Peptide Set (Cat. No. 67171K) prior to antibody probing steps (lane 2).



Western blot analysis of Bcl-X in K652 leukemia cell lysate. Lane 1, clone 2H12 (Cat. No. 66461A) detects Bcl-X as an ~29 kD band. To demonstrate specificity, the binding of clone 2H12 to the Bcl-X protein was blocked by preincubation with 2H12 specific blocking peptide which is part of the Bcl-X (2H12) Blocking Peptide Set (Cat. No. 68001K) prior to antibody probing steps (lane 2).

Description	Clone(s)	Specificity	Apps.	Format	Size	Cat. No.
Bad	P3F6	Hu	WB, IHCP	PU	0.1 mg	66551A
Bad	2G11	Ms	IP, WB	PU	0.1 mg	13361A
Bak	Polyclonal	Hu	WB	SE	0.1 ml	66026E
Bak	G317-1		WB, IHCF	PU	0.1 mg	65401A
Bak	G317-2	Hu	WB	PU	0.1 mg	65371A
Bax	6A7	Hu, Ms, R	IP, WB	PU	0.1 mg	66241A
Bax	G206-1276	Hu, Ms	IP, WB, IHCF, IHCP	PU	0.1 mg	13401A
Bax	Polyclonal	Hu	IP, WB, IHCF, IHCP	SE	0.1 ml	13666E
Bax	Polyclonal	Ms, R	IP, WB, IHCF, IHCP	SE	0.1 ml	13686E
Bax	4D2	Ms	IP	PU	0.1 mg	13421A
Bcl-2	Polyclonal	Hu	IP, WB, FC, IHCF, IHCP	SE	0.1 ml	14371E
Bcl-2	Bcl-2/100	Hu	WB	PU	0.1 mg	65111A
Bcl-2 Antibody Reagent Set	Bcl-2/100, MOPC-21	Hu	FC	F Set	100 tests	6511KK
			FC	PE Set	100 tests	6681KK
Bcl-2	4D7	Hu	IP, WB	PU	0.1 mg	14831A
Bcl-2	6C8	Hu	IP, WB, FC, IHCF	PU	0.1 mg	15131A
Bcl-2 Antibody Reagent Set	6C8, Ha4/8	Hu	FC	F Set	100 tests	1513KK
			FC	PE Set	100 tests	6682KK
Bcl-2	Polyclonal	Ms	IP, WB, IHCF, IHCP	SE	0.1 ml	15616E
Bcl-2 Antibody Reagent Set	3F11, A19-3	Ms	FC	F Set	100 tests	1502KK
			FC	PE Set	100 tests	6683KK
Bcl-2	Polyclonal	Ms, R	IP, WB, IHCF, IHCP	SE	0.1 ml	13456E
Bcl-X	Polyclonal	Hu, Ms	WB, IHCF, IHCP	SE	0.1 ml	65186E
Bcl-X	2H12	Hu, Ms, R	WB, IHCP	PU	0.1 mg	66461A

Related Reagents

Bax (6A7) Blocking Peptide Set*	EBS	PEP Set	0.1 mg ea	67171K
Bcl-X (2H12) Blocking Peptide Set*	EBS	PEP Set	0.1 mg ea	68001K

*Each set contains 0.1 mg specific blocking peptide and 0.1 mg nonspecific blocking peptide.

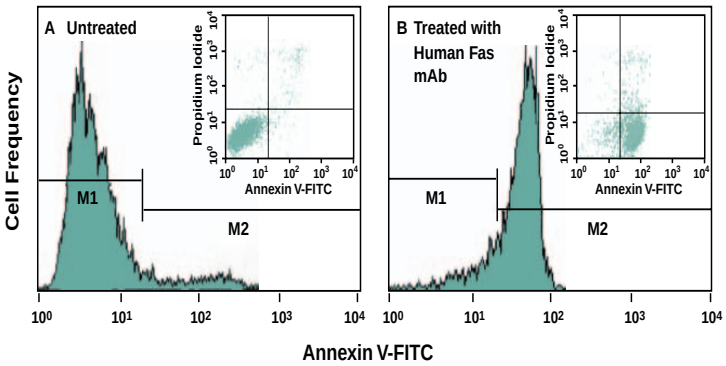
Apoptosis Detection Kits

Annexin V Reagents and Kits

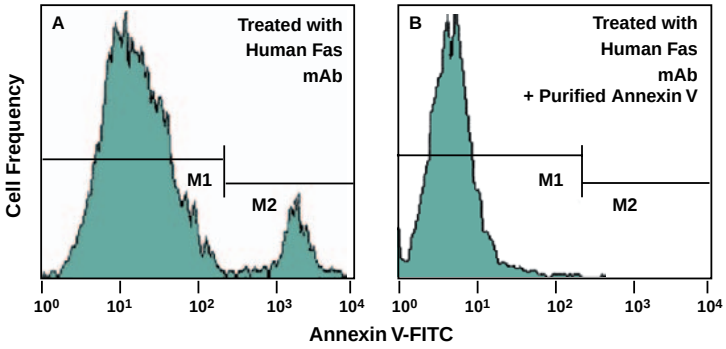
Apoptosis is characterized by a variety of morphological features. Changes in the plasma membrane are one of the earliest of these features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kD Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes such as FITC and PE, or to Biotin. These formats retain their high affinity for PS and thus serve as sensitive probes for flow cytometric analysis of cells that are undergoing apoptosis.

Because externalization of PS occurs in the earlier stages of apoptosis, Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with Annexin V in conjunction with vital dyes such as Propidium iodide (PI) or 7-amino-actinomycin D (7-AAD) allows the investigator to identify early apoptotic cells (Annexin V positive, PI negative). The assay does not identify between cells that have already undergone an apoptotic death and those that have died as a result of a necrotic pathway, because in either case the dead cells will stain with both Annexin V (positive) and PI (positive).

PharMingen offers Annexin V reagents individually or conveniently packaged in our Annexin V-FITC Apoptosis Detection Kits I and II. These reagents are flow cytometry based for quantitative analysis of apoptotic cells.



Flow cytometric analysis of apoptotic cells using Annexin V-FITC. HBP-ALL leukemia cells were left untreated (A) or treated for 2 hr (B) with anti-human Fas antibody, clone DX2 (Cat. No. 33450D) and Protein G*. Cells were incubated with Annexin V-FITC (Cat. No. 65874X or 65874H) in a buffer containing Propidium iodide (PI), (Cat. No. 66211E) and analyzed by flow cytometry. Untreated cells were primarily Annexin V-FITC and PI negative (inset A), indicating that they were viable and not undergoing apoptosis. After a 2 hr treatment with DX2, the majority of cells were undergoing apoptosis (Annexin V-FITC positive and PI negative; inset B). The M1 and M2 gates demarcate Annexin V-FITC negative and positive populations, respectively.



Flow cytometric analysis of Annexin V-FITC staining and blocking with recombinant Annexin V. Jurkat T cells were induced to undergo apoptosis by treatment with anti-human Fas antibody, clone DX2 (Cat. No. 33450D) and Protein G* for 3 hr. Cells were then incubated with Annexin V-FITC (Cat. No. 65874X or 65874H) alone (A) or with Annexin V-FITC in the presence of recombinant Annexin V (Cat. No. 65871A) (B) to block Annexin V-FITC binding sites, thus demonstrating the specificity of Annexin V-FITC staining.

*The addition of Protein G enhances the ability of DX2 to induce apoptosis, presumably by cross-linking Fas receptors.

Description	Size	Cat. No.
Annexin V-FITC Detection Kit I	100 tests	6693KK

Kit Components

Annexin V-FITC	100 tests
Annexin V Binding Buffer	50 ml
Propidium Iodide Solution	2.0 ml

Description	Size	Cat. No.
Annexin V-FITC Detection Kit II	100 tests	6710KK

Kit Components

Annexin V-FITC	100 tests
Recombinant Annexin V	100 µg
Annexin V Binding Buffer	50 ml
Propidium Iodide Solution	2.0 ml

Description	Apps.	Format	Size	Cat. No.
Recombinant Annexin V (For Blocking)	FC	Unlabeled	0.1 mg	65871A
Annexin V-Biotin	FC	Biotin	200 tests	65872H
Annexin V-Biotin	FC	Biotin	100 tests	65872X
Annexin V-FITC	FC	FITC	200 tests	65874H
Annexin V-FITC	FC	FITC	100 tests	65874X
Annexin V-PE	FC	PE	200 tests	65875H
Annexin V-PE	FC	PE	100 tests	65875X
Annexin V-FITC Detection Kit I	FC	Kit	100 tests	6693KK
Annexin V-FITC Detection Kit II	FC	Kit	100 tests	6610KK
Annexin V Binding Buffer	FC	Buffer	50 ml	66121E
Propidium iodide Solution	FC	Buffer	2.0 ml	66211E
ViaProbe™ (7-AAD) Staining Solution	FC	Buffer	100 tests	34321X

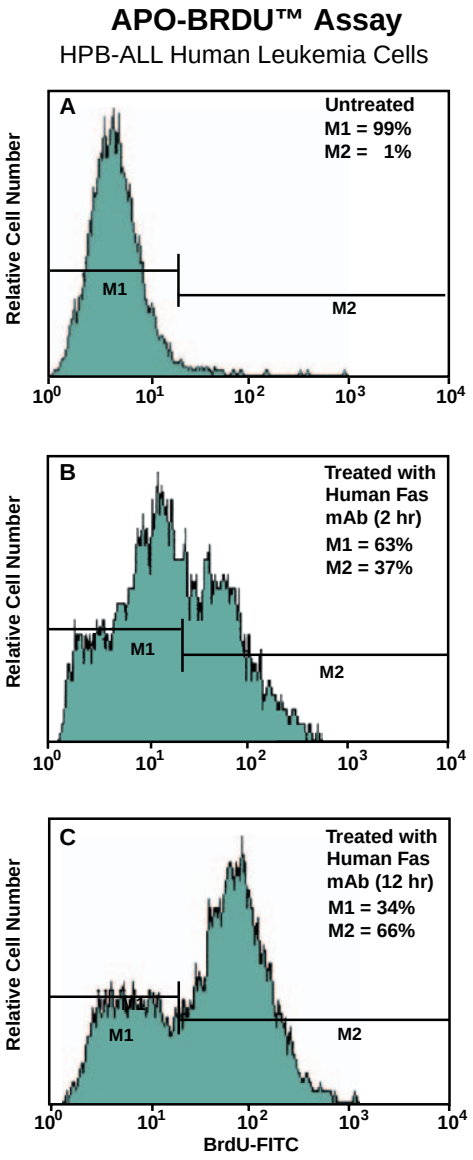
Apoptosis Detection Kits

APO-DIRECT™ and APO-BRDU™ Kits

One of the later steps in apoptosis is DNA fragmentation, a process which begins with the activation of endonucleases which become activated during the apoptotic program. These nucleases degrade the higher order chromatin structure into fragments of 50 to 300 kb and subsequently into smaller DNA pieces of about 200 bp length. A method which is often used to detect fragmented DNA utilizes a reaction catalyzed by exogenous TdT, referred to as "end-labeling" or "TUNEL" staining.

APO-BRDU™: The enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes a template independent addition of bromolated deoxyribonucleotide triphosphates (Br-dUTP) to the 3'-hydroxyl (OH) termini of double- and single-stranded DNA. It has been shown that Br-dUTP is more readily incorporated into the genome of apoptotic cells than are the deoxyribonucleotide triphosphates complexed to larger molecules like fluorescein, biotin or digoxigenin. After incorporation, these sites are identified by flow cytometric means by staining the cells with a FITC-labeled anti-BrdU mAb.

APO-DIRECT™: The enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes a template independent addition of FITC-labeled deoxyribonucleotide triphosphates (FITC-dUTP) to the 3'-hydroxyl (OH) termini of double- and single-stranded DNA. The Apo-DIRECT™ assay is therefore a single-step method for labeling DNA breaks with FITC-dUTP followed by flow cytometry analysis.



APO-BRDU™ Kit, Cat. No. 6576KK. HBP-ALL human leukemia cells were left untreated (A) or treated with anti-human Fas mAb, clone DX2 (Cat. No. 33450D), and Protein G* for 2 hr (B) or 12 hr (C). Cells were fixed and incubated with Br-dUTP** in the presence of TdT enzyme** in order to incorporate Br-dUTP into exposed 3'-OH DNA ends. Br-dUTP was detected with a fluorescein labeled anti-BrdU mAb**. Nonapoptotic cells (M1 gates) do not incorporate significant amounts of Br-dUTP due to lack of exposed 3'-OH DNA ends, and consequently have relatively little fluorescence compared to apoptotic cells which have an abundance of 3'-OH ends (M2 gates). DX2 induced Fas-mediated apoptosis is shown by an increase in the number of cells staining with anti-BrdU-FITC mAb (M2 gates) after 2 and 12 hr. The M1 and M2 gates demarcate non-apoptotic and apoptotic populations, respectively.

*The addition of Protein G enhances the ability of DX2 to induce apoptosis, presumably by cross-linking Fas receptors.

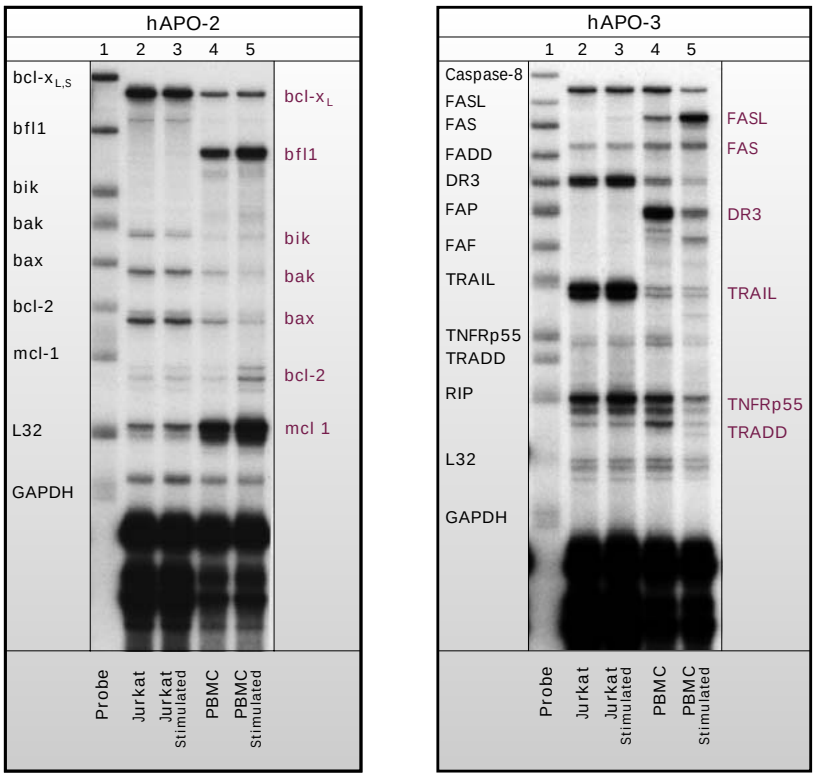
***Components of the APO-BRDU™ Kit.*

Description	Apps.	Format	Size	Cat. No.
APO-BRDU™ KIT	FC	Kit	60 tests	6576KK
APO-DIRECT™ KIT	FC	Kit	50 tests	6536KK

RiboQuant® Multi-Probe Ribonuclease Protection Assay System

The RiboQuant® Multi-Probe RNase Protection Assay can be used to measure mRNA levels of molecules participating in apoptosis during development, homeostasis, and in many disease conditions. The multi-probe ribonuclease protection assay (RPA) is a method for detecting and quantifying the expression of multiple genes in a single RNA sample. This assay system involves the hybridization of the RNA of interest to their complementary antisense RNA probes. Each individual apoptosis, cell cycle, tumor suppressor, and stress protein gene specific template has been assembled into relevant sets, to be used by investigators for the T7 RNA polymerase-directed synthesis of a high-specific activity, α^{32}P-labeled, anti-sense RNA probe set (Tables 1-3). In each set, we have included the L32 gene (which encodes a ribosomal protein) and the GAPDH gene to serve as housekeeping gene controls. Following transcription using the RiboQuant® *In Vitro* Transcription Kit (Cat. No. 45004K) the probe set and total target RNA are solution hybridized overnight. After overnight hybridization utilizing the reagents included in the RPA Kit (Cat. No. 45014K), free probe and single-stranded non-protected RNA molecules are digested with a pre-optimized mixture of RNase A and T1. The remaining RNase-protected probes are treated with Proteinase K, extracted, precipitated, washed and loaded onto a 5% denaturing polyacrylamide gel. The resulting resolved bands are separated on the gel according to their size, and imaged by autoradiography, beta-scanning or phosphorimaging systems.

Utility of the RPA for Analyzing RNA from Cell Populations



Autoradiograms of protection assays utilizing hAPO-2 and hAPO-3 multi-probe template sets with RNA isolated from Jurkat T cells and Human PBMCs. Jurkat cells and human PBMCs were cultured in the presence or absence of 1 µg/ml of ionomycin for 3.5 hr. Total cellular RNA was isolated and treated according to protocol using hAPO-2 (Cat. No. 45354P; bcl-2 related molecules) and hAPO-3 (Cat. No. 45355P; death receptor signaling molecules). The profile from PBMCs of expressed RNAs was consistent with activated and proliferating cells. Characteristic increases in the level of anti-apoptotic messages were seen for bcl-2, bcl-x, bfl-1, mcl-1, and FAP. The levels of transcripts coding for molecules responsible for causing death were generally reduced (see bak, bax, DR3, TNFRp55, and TRADD). One exception to this is the increase in FasL expression. An upregulation in FasL levels upon activation has been previously reported. The combination of reduction in death-inducing molecules and the increase in those which prevent death shifts the balance in the cell to a state in which it can grow and proliferate rather than die.

Table 1a. Human Apoptosis Multi-Probe Sets

hAPO-1b	hAPO-1c	hAPO-2	hAPO-2c	hAPO-3	hAPO-3b	hAPO-3c	hAPO-4	hAPO-5
Caspase-8	Caspase-8	-	bcl-w	Caspase-8	Caspase-8	Caspase-8	Granzyme A	XIAP
Granzyme B	Caspase-4	bcl-X_L	bcl-X_L	FASL	FASL	FASL	Granzyme B	TRAF1
Caspase-3	Caspase-3	bcl-X_S	bcl-X_S	FAS	FAS	FAS	DAD1	TRAF2
Caspase-6	Caspase-6	bfl1	bfl 1	FADD	CLARP	DCR1	FAST K	TRAF4
-	Caspase-10a	-	bad	DR3	FAP	DR3	Granzyme H	NAIP
Caspase-5	Caspase-5	bik	bik	FAP	CRADD	DR5	RVP1	-
Caspase-2 (S)	Caspase-2 (S)	bak	bak	FAF	Daxx	DR4	Dr-nm23	c-IAP-2
Caspase-7	Caspase-7	bax	bax	TRAIL	MADD	TRAIL	Granzyme 3	c-IAP-1
Caspase-1	Caspase-1	bcl-2	bcl-2	TNFRp55	-	TNFRp55	Requiem	TRPM2
Caspase-2 (L)	Caspase-2 (L)	mcl-1	mcl-1	TRADD	-	TRADD	CAS	CRAF
-	Caspase-9	-	-	RIP	RIP	RIP	perforin	-
Caspase-9	-	-	-	-	-	-	-	-
L32	L32	L32	L32	L32	L32	L32	L32	L32
GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH

Table 1b. Human Apoptosis Multi-Probe Sets

hAPO-5b	hAPO-5c	hAPO-6
-	XIAP	IPL
TRAF1	survivin	ASK1
TRAF2	-	Harakiri
CART	-	SIAH
I-TRAF	NAIP	DFF
TRAF5	-	Nip2
TRAF6	c-IAP-2	Nip3
-	c-IAP1	Nip1
-	TRPM-2	DAP-Kinase
CRAF	-	DAP
TRIP	-	DRM
-	-	-
L32	L32	L32
GAPDH	GAPDH	GAPDH

Table 2. Mouse Apoptosis Multi-Probe Sets

mAPO-1	mAPO-2	mAPO-3
Caspase-8	bcl-w	Caspase-8
-	bfl1	FASL
Caspase-3	bcl-X_L	FAS
Caspase-6	bcl-X_S	FADD
Caspase 11	-	-
Caspase 12	-	FAP
Caspase-2 (L)	bak	FAF
-	-	-
Caspase-7	bax	TRAIL
Caspase-1	bcl-2	TNFRp55
Caspase X	-	TRADD
Caspase-2 (S)	-	RIP
-	bad	-
L32	L32	L32
GAPDH	GAPDH	GAPDH

Table 3. Rat Apoptosis Multi-Probe Set

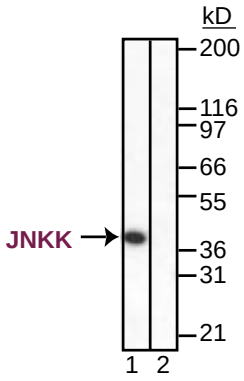
rAPO-1
FAS
bcl-X_L
bcl-X_S
FASL
Caspase-1
Caspase-8
Caspase-2 (L)
-
bax
bcl-2
-
Caspase-2 (S)
-
L32
GAPDH

Description	Specificity	Apps.	Format	Size	Cat. No.
RiboQuant® *In Vitro* Transcription Kit		IVT	Kit	25 RXNS	45004K
RiboQuant® RNase Protection Assay Kit		RPA	Kit	200 RXNS	45014K
RiboQuant® RPA Starter Package (includes RPA Kit, *In Vitro* Transcription Kit, and 1 Probe Set)		RPA	Kit		45024K
hAPO-1b RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45378P
hAPO-1c RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45607P
hAPO-2 RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45121P
hAPO-2c RiboQuant®Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45609P
hAPO-3 RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45131P
hAPO-3b RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45611P
hAPO-3c RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45608P
hAPO-4 RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45141P
hAPO-5 RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45151P
hAPO-5b RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45610P
hAPO-5c RiboQuant® Apoptosis Multi-Probe Template Set	Hu	RPA	Template Set	10 RXNS	45613P
hAPO-6 RiboQuant® Apoptosis Multi-Probe Template set	Hu	RPA	Template Set	10 RXNS	45612P
mAPO-1 RiboQuant® Multi-Probe Template Set	Ms	RPA	Template Set	10 RXNS	45358P
mAPO-2 RiboQuant® Apoptosis Multi-Probe Template Set	Ms	RPA	Template Set	10 RXNS	45354P
mAPO-3 RiboQuant® Apoptosis Multi-Probe Template Set	Ms	RPA	Template Set	10 RXNS	45355P
rAPO-1 RiboQuant® Apoptosis Multi-Probe Template Set	R	RPA	Template Set	10 RXNS	45601P
HeLa Control RNA	Hu	RPA	Template Set	10 RXNS	45201Z
Control RNA-2	Ms	RPA	Template Set	10 RXNS	45382Z
Control RNA	R	RPA	Template Set	10 RXNS	45216Z

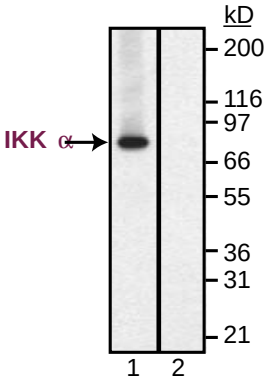
Other Apoptosis-Related Proteins

Signal Transducers

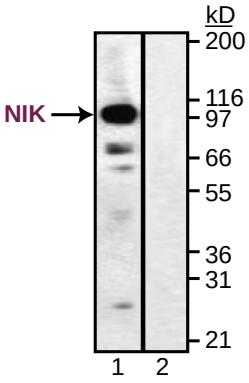
Apoptosis is one of a number of phenotypic responses that may occur as a result of signal transduction pathways in the cell. Clustering of cellular receptors is a commonly observed first step in the mechanism of signal transduction pathways which result in apoptosis. Clustered receptor cytoplasmic domains trigger a variety of signal tranducing proteins. These proteins are critical mediators of apoptotic pathways beginning at the cell surface, where they may participate directly within a receptor signal complex, to the nucleus and the activation of genes required during the apoptotic process.



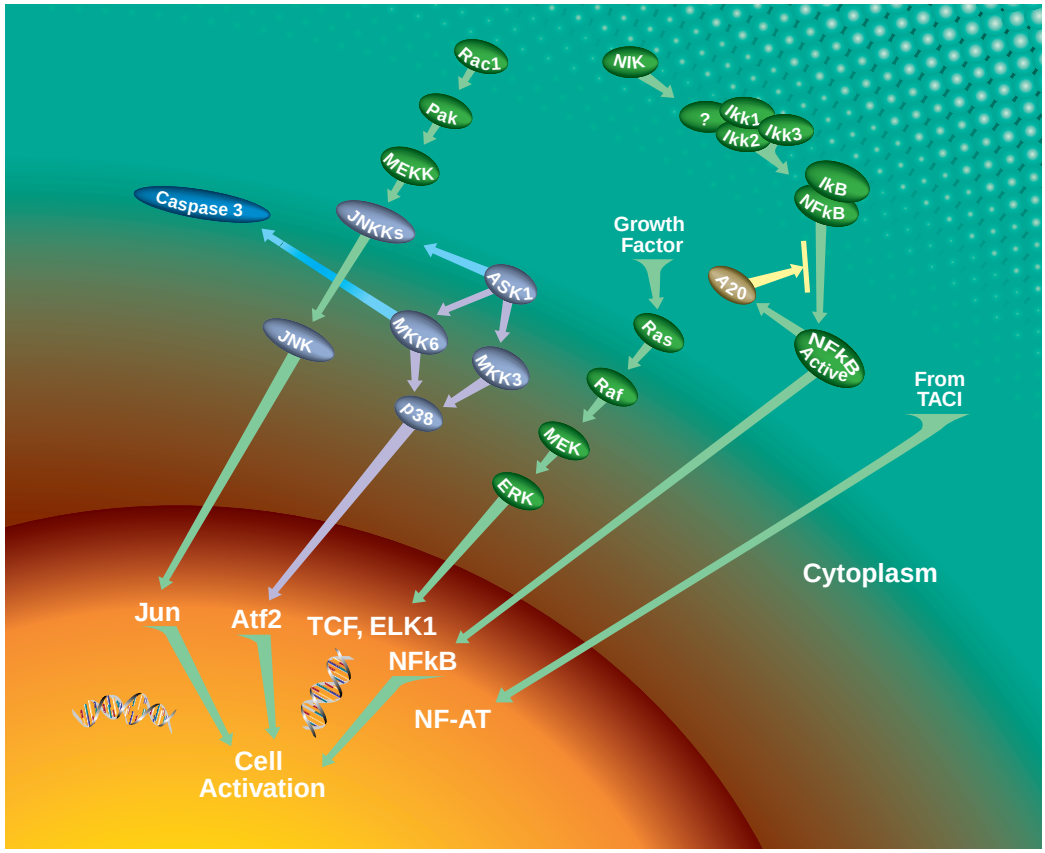
Western blot analysis of JNKK in HeLa human carcinoma cell lysates. Lane 1, clone A32-1 (Cat. No 65731A). Lane 2, mouse IgG_1 isotype control. Clone A32-1 identifies JNKK as an ~45 kD band.



Western blot analysis of IKKα in Daudi B lymphoma cell lysate. Lane 1, anti-IKKα, clone B78-1 (Cat. No 66781A). Lane 2, a mouse IgG_{2b} isotype control. Clone B78-1 identifies IKKα as an ~85 kD band.



Western blot analysis of NIK in 293 embryonic kidney cell lysate. Affinity purified, rabbit anti-human NIK (Cat. No. 67091N) identifies NIK as an ~104 kD band.

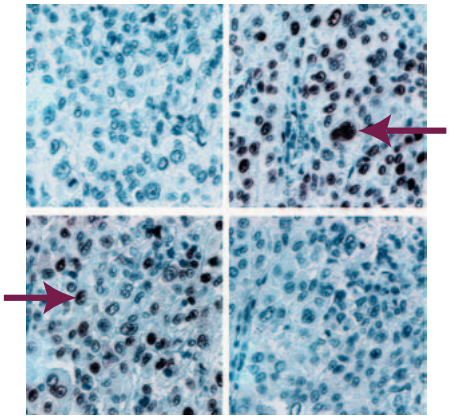


Description	Clone(s)	Specificity	Apps.	Format	Size	Cat. No.
ASK1	Polyclonal	Hu	WB	PU	50 µg	68031N
IKKα	B78-1	Hu	IP/WB	PU	0.1 mg	66781A
JNK1	G151-333	Hu	IP, WB, IVK	PU	0.1 mg	15701A
JNK1	G151-666	Hu	IP, WB	PU	0.1 mg	15691A
JNKK	G282-114	Hu	IP, WB	PU	0.1 mg	13671A
JNKK	A32-1	Hu	IP, WB	PU	0.1 mg	65731A
MEK1	Polyclonal	Hu, Ms	WB	SE	0.1 ml	13586E
MEK2	A7-1	Hu, Ms	WB	PU	0.1 mg	65421A
MEK2	Polyclonal	Hu, Ms	WB	SE	0.1 ml	13596E
MEKK1	Polyclonal	Hu, Ms	WB	SE	0.1 ml	66866E
c-myc	9E10	Hu	WB, IHCP	PU	0.1mg	14851A
N-myc	B8.4.B	Hu	IP/WB	PU	0.1 mg	66001A
N-myc	N-Myc-2	Hu, Ms	IP, WB	PU	0.1 mg	14861A
NIK	Polyclonal	Hu	IP/WB	PU	50 µg	67091N

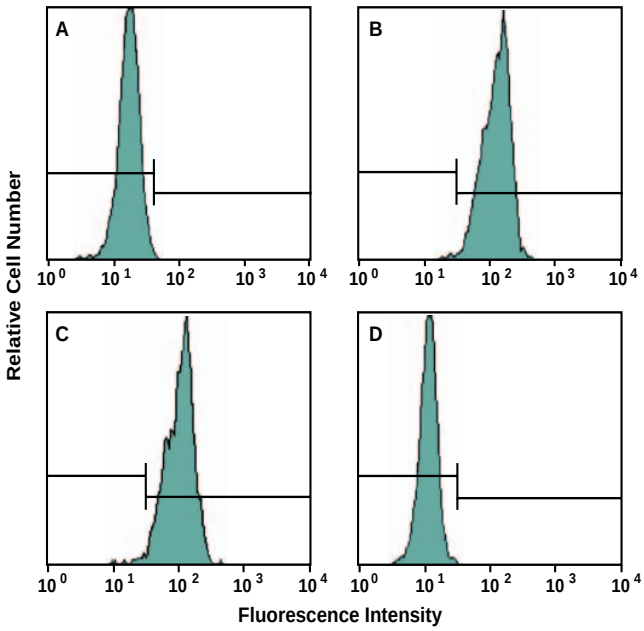
Other Apoptosis-Related Proteins

Tumor Suppressors

Tumor Suppressor genes normally function to regulate and restrict cell proliferation. Many tumor suppressors are components of intracellular signalling pathways that enable a cell to receive and process growth-inhibitory signals from its surroundings. When a cell loses expression of critical components of the signalling network, it can lose its ability to respond to certain extracellular growth-inhibitory signals and proliferate. Growth deregulation resulting from the absence of normal expression or function of a tumor suppressor gene can then lead to uncontrolled cell proliferation and tumor formation.



Immunohistochemical staining of Rb in human osteosarcoma tissue sections. Tissues were stained with a mouse IgG isotype control (upper left) or with clone G3-245 (upper right). To demonstrate specificity, clone G3-245 was preincubated with either a nonspecific (negative control) peptide (lower left) or with a G3-245 specific blocking peptide (lower right). Arrows indicate positive staining with G3-245.

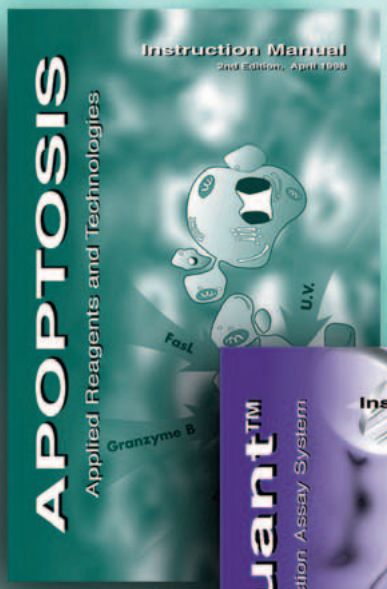


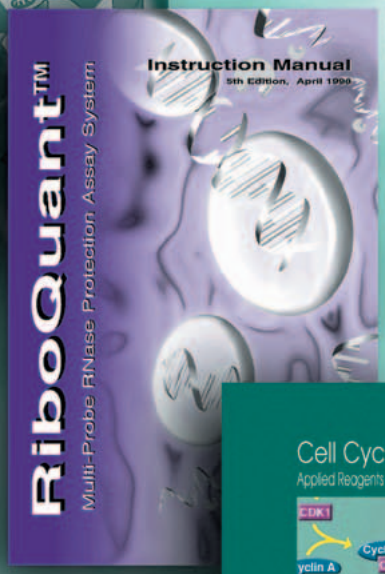
Flow cytometric analysis of Rb in permeabilized MOLT4 leukemia cells. Cells were stained with a mouse IgG isotype control (A) or with clone G3-245, anti-Rb-PE Antibody Reagent Set (Cat. No. 6685KK) (B). To demonstrate specificity, anti-Rb-PE was preincubated with either a G3-245 nonspecific blocking peptide (C) or with a specific peptide (D).

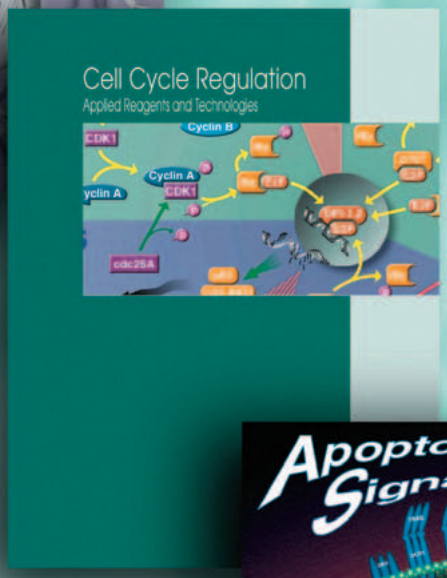
Description	Clone(s)	Specificity	Apps.	Format	Size	Cat. No.
p33^{ING}	Polyclonal	Hu, Ms	WB, IF	SE	0.1 ml	66156E
p53	G59-12	Hu, Ms, R	IP, WB, IHCF, IHCP	PU	0.1 mg	14211A
p53	PAb 122	Hu, Ms, R	IP, WB, FC	PU	0.1 mg	14091A
p53	PAb 240	Hu, Ms, R	IP, WB, IHCF	PU	0.1 mg	14461A
			IP, WB,IHCF	PU	0.25 mg	14461C
p53	DO-1	Hu	IP, WB, IHCF, IHCP	PU	0.1 mg	15791A
p53	DO-7	Hu	IP, WB, FC, IHCF, IHCP	PU	0.1 mg	15801A
p53 Antibody Reagent Set	DO-7,27-35	Hu	FC	F Set	100 tests	1580KK
				PE Set	100 tests	6680KK
p53	PAb 1801	Hu	IP, WB, IHCF, IHCP	PU	0.1 mg	14471A
				PU	0.25 mg	14471C
p53	PAb 246	Ms	IP	PU	0.1 mg	14451A
Rb Antibody Reagent Set	G3-245, MOPC-21	Hu	FC	F Set	100 tests	6684KK
Rb			FC	PE Set	100 tests	6685KK
Rb	G4-340	Hu	IP, WB, IHCF	PU	0.1 mg	14021A
Rb	G3-349	Hu	IP, WB, IHCF	PU	0.1 mg	14011A
Rb	C36	Hu	IP, WB, IHCF	PU	0.1 mg	14031A
Rb	G99-73	Hu	WB	PU	0.1 mg	14421A
Rb	G99-549	Hu	IP, WB	PU	0.1 mg	14441A
Rb	G99-2005	Hu	IP, WB	PU	0.1 mg	14411A
Rb	XZ55	Hu	IP, WB	PU	0.1 mg	14051A
Rb	XZ91	Hu	IP, WB, IF	PU	0.1 mg	14061A
Rb	XZ104	Hu	IP	PU	0.1 mg	14041A
Rb	XZ133	Hu	IP	PU	0.1 mg	14071A
β-Amyloid (a.a. 1-40)	Polyclonal	Hu	E, IHCF	SE	25 µg	66471G
β-Amyloid (a.a. 1-42)	Polyclonal	Hu	E, IHCF	SE	25 µg	66481G
β-Amyloid (a.a. 1-43)	Polyclonal	Hu	E, IHCF	SE	25 µg	66491G
Related Reagents						
p53 (PAb 122) Blocking Peptide Set*			EBS	PEP Set	0.1 mg ea	67111K
p53 (DO-7) Blocking Peptide Set*			EBS	PEP Set	0.1 mg ea	67141K
Rb (G3-245) Blocking Peptide Set*			EBS	PEP Set	0.1 mg ea	66671K
β-Amyloid (a.a. 1-40)		Hu	EBS	PEP	250 µg	66501C
β-Amyloid (a.a. 1-42)		Hu	EBS	PEP	250 µg	66511C
β–Amyloid (a.a. 1-43)		Hu	EBS	PEP	250 µg	66521C

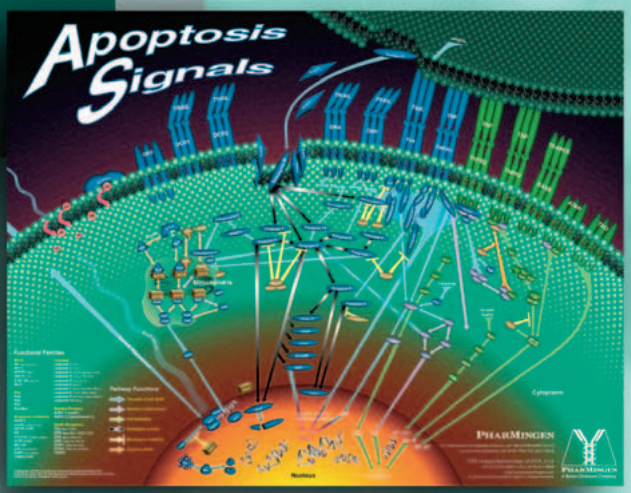
*Each set contains 0.1 mg specific blocking peptide and 0.1 mg nonspecific blocking peptide.

PharMingen Apoptosis Related Literature









- Manuals
- Mailers
- Poster
- Slide Sets

Please call or visit our web site to request any or all of these items

Orders 877.232.8995 • Phone 858.812.8800
World Wide Web http://www.bdbiosciences.com

Pharmingen International

Asia Pacific
BD Singapore
Tel (65) 860-1478
Fax (65) 860-1590

Europe
PharMingen Europe
Tel (49) 40 532 84 48 0
Fax (49) 40 531 58 92

Japan
Fujisawa Pharmaceutical Co.,Ltd.
Tel (81) 3 5256-5311
Fax (81) 3 5256-5370

Canada
PharMingen Canada
Toll-Free 1-888-259-0187
Tel 905-542-8028
Fax 905-542-9391
e-mail: canada@pharmingen.com

United States
Pharmingen
Tel 858-812-8800
Orders 877.232.8995
Tech Service 877.232.8995
Fax 619-812-8889
http://www.bdbiosciences.com

PHARMINGEN
A Becton Dickinson Company

Africa
Becton Dickinson Worldwide Inc.
Tel (254) 2 449 608
Fax (254) 2 449 619

Australia
Becton Dickinson Pty Ltd
Tel (612) 9978-6800
Fax (612) 9978-6850

Austria
Becton Dickinson GmbH
HQ PharMingen Europe
Tel (49) 40 532 84 48 0
Fax (49) 40 531 58 92

Belgium
Becton Dickinson Benelux N.V.
Immunocytometry Systems
Orders
Tel (32) 53 72 05 50
Fax (32) 53 72 05 49
Technical Service
Tel (32) 53 72 06 08
Fax (32) 53 72 06 20

China
Becton Dickinson Beijing
Tel (86-10) 6593-3072
Fax (86-10) 6593-3070

Denmark
Becton Dickinson
Tel (45) 43 43 45 66
Fax (45) 43 43 41 66

Finland
Oriola Oy Prolab
Tel (358) 9 429 99
Fax (358) 9 429 2080

France
Becton Dickinson
Immunocytometry Systems
Orders
Tel (33) 476 68 37 32
Fax (33) 476 68 35 06
Technical Service
Tel (33) 476 68 34 25
Fax (33) 476 68 35 06

Germany
Becton Dickinson GmbH
HQ PharMingen Europe
Tel (49) 40 532 84 48 0
Fax (49) 40 531 58 92

Greece
Becton Dickinson Hellas S.A.
Tel (30) 1 9307741
Fax (30) 1 9307740

Hong Kong
Becton Dickinson Asia Ltd
Tel (852) 2572-8668
Fax (852) 2520-1837

Hungary
Soft Flow Hungary kft.
Tel/Fax (36) 72 240064

India
Becton Dickinson Asia Ltd
Tel (91-11) 6830987
Fax (91-11) 6831783/5403

Indonesia
Becton Dickinson Asia Ltd
Tel (62-21) 5480494
Fax (62-21) 5480686

Israel
Enco Scientific
Tel (972-3) 934-9922
Fax (972-3) 934-9876

Italy
Immucor Italia
Tel (39-2) 576-04555
Fax (39-2) 576-00378

Korea
Becton Dickinson Korea Inc
Tel (822) 5694030
Fax (822) 5694048/9

Latin/South America
BDIS (USA)
Tel (408) 954-2157
Fax (408) 526-1804

Malaysia
Becton Dickinson Sdn Bhd
Tel (03) 7571323
Fax (03) 7571153

Mexico
Becton Dickinson de Mexico
Tel (52-5) 237-12-00
Fax (52-5) 237-12-87

Middle East
Becton Dickinson
Tel (971) 4 379525
Fax (971) 4 379551

The Netherlands
Becton Dickinson B.V.
Immunocytometry Systems
Orders
Tel (31) 76 50 12530
Fax (31) 76 50 12830
Technical Service
Tel (31) 76 50 38595
Fax (31) 76 50 12830

Norway
LABOREL A/S
Tel (47) 23 05 19 30
Fax (47) 22 63 07 51

Phillipines
Becton Dickinson Phillipines, Inc.
Tel (632) 8135275
Fax (632) 8105687

Poland
Becton Dickinson Polska Sp.z.o.o.
Tel (48) 22 651 7921, -22, -23
Fax (48) 22 651 7924

Portugal
ENZIfarma Diagnóstica e Farmaceutica, Lda.
Tel (351) 1 422 0100
Fax (351) 1 422 0110

South Africa
Becton Dickinson
Immunocytometry Systems
Tel (27) 11 807 1531
Fax (27) 11 807 1953

Spain
Becton Dickinson S.A.
Tel (34) 1 848 8100
Fax (34) 1 848 8105
Orders
Tel (34) 1 848 8182
Fax (34) 1 848 8104

Sweden
Becton Dickinson AB
Tel (46) 8 775 51 00
Fax (46) 8 645 08 08

Switzerland
Becton Dickinson GmbH
HQ PharMingen Europe
Tel (49) 40 532 84 48 0
Fax (49) 40 531 58 92

Taiwan
Becton Dickinson Worldwide Inc
Tel (886) 722-5660
Fax (886) 725-1772

Thailand
Becton Dickinson Thailand Ltd
Tel (662) 2607370-5
Fax (662) 2607377

Turkey
Becton Dickinson Turkey
Tel (90) 212 222 87 77
Fax (90) 212 222 87 76

United Kingdom/Eire
Becton Dickinson UK Ltd.
Orders:
Tel (44) 800 783 0373
Technical Service:
Tel (44) 800 783 0342
Fax (44) 1865 781 635