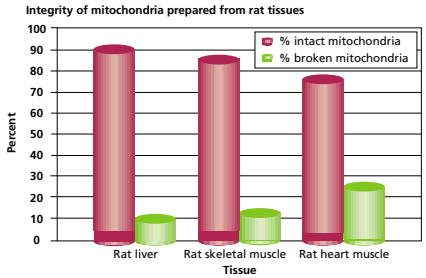
Apoptosis Detection

Kits

CYTOC-OX1 **Cytochrome c Oxidase Assay Kit**
Sufficient for 100 tests

For the determination of cytochrome c oxidase activity in mitochondrial soluble and membrane bound samples. Cytochrome c oxidase [EC 1.9.3.1.] is present in mitochondria of the more highly developed cells and in the cytoplasmic membrane of bacteria. This enzyme provides energy for the cell by coupling electron transport through the cytochrome chain with the process of oxidative phosphorylation. Cytochrome c oxidase is located on the inner mitochondrial membrane that divides the mitochondrial matrix from the intermembrane space and traditionally it has been used as a marker for this membrane. The colorimetric assay measures the decrease in absorbance of ferrocytochrome c caused by its oxidation to ferricytochrome c by cytochrome c oxidase.



Cytochrome c Oxidase activity is measured in the presence and absence of the detergent 1 mM n-dodecyl maltoside. The ratio of the two activities provides a measure of the integrity of the outer mitochondrial membrane.

PCR Primer Sets

APO-PCR **Apoptosis PCR Bax/Bcl-2 Multiplex Primer Sets**
Sufficient for 50 (50 µl) PCR reactions

The kit is useful for studying the progress of apoptosis and the evaluation of potential apoptosis inducers.
Bcl-2 is a member of a large gene family encoding proteins that can either inhibit (e.g. Bcl-2, Bcl-X_L) or promote (e.g. Bax, Bcl-X_S, Bak) apoptosis. Bcl-2 inhibits apoptosis by preventing the release of apoptosis inducing factor (AIF) and cytochrome c from the mitochondria. If the Bcl-2 level is higher than the Bax level, apoptosis will be inhibited. The ratio of Bcl-2 to Bax in the cell can determine whether or not the cell initiates apoptosis or survives. Some cancer cells such as melanoma overexpress Bcl-2 preventing apoptosis and allowing malignant growth to continue.
The kit contains primer sets for the detection of Bax and Bcl reverse transcribed from mRNA from rat, mouse or human tissues or cell lines (reverse transcriptase and thermostable DNA polymerase not included). A primer set for a "housekeeping" gene, GAPDH, as an internal control is included in the kit. These primer sets are optimized for the simultaneous amplification, detection and comparison of Bax and Bcl-2 mRNA levels. The sizes of the amplified products are 487bp for Bax, 127bp for Bcl-2 and 349bp for GAPDH.

Features and Benefits:

- Simultaneous RT-PCR amplification and detection of Bax and Bcl-2 mRNA levels.
- Includes primers for the GAPDH gene as an internal control for comparison of Bax and Bcl-2 mRNA levels to allow for semiquantitative evaluation of Bax and Bcl-2.
- Functions with Taq DNA polymerase, AccuTaq™ LA DNA polymerase, REDTaq™ DNA polymerase, and equivalent DNA polymerases.
- Primer solutions contain both sense and anti-sense primers of the specific gene for convenience.
- The primers work with cDNA reverse transcribed from RNA (mRNA and total RNA) extracted from cells and tissues using various extraction methods.
- Works in one step RT-PCR procedures.
- Genomic DNA contamination in the RNA will not be amplified.

Apoptosis PCR Primer Sets
Sufficient for 50 reactions

Synthetic oligonucleotide primers for RT-PCR analysis of mRNA expression. Amplifies reverse-transcript mRNAs from human, mouse and rat sources. Set includes an upstream and downstream oligonucleotide.

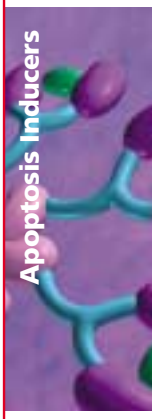
B 3055 **Bad PCR Primer Set**
B 3180 **Bak PCR Primer Set**
B 8304 **Bax PCR Primer Set**
B 9179 **Bcl-2 PCR Primer Set**
F 8425 **Fas PCR Primer Set**
F 8300 **Fas-Ligand PCR Primer Set**

Apoptosis Detection

Reagents		
T 4427	**Terminal Transferase** **From calf thymus** (Terminal Deoxynucleotidyl Transferase: TdT) ≥5,000 units/mL Buffered aqueous glycerol solution	TdT is a primer-dependent polymerase that catalyzes the addition of deoxynucleotides to the 3′-OH terminus of DNA molecules with the release of inorganic phosphate. TdT reacts preferentially with either single-stranded DNA molecules or double-stranded-DNA with 3′ overhangs, but procedures have been developed to label blunt ends or 3′-recessive ends. In a reaction mixture, the divalent ion (Co^{2+}, Mn^{2+}, Mg^{2+}) will influence purine and pyrimidine polymerization rate. Activities of TdT are also affected by the bases (dATP, dCTP, dGTP and dTTP) present. Each reaction mixture needs to be optimized. The enzyme is commonly used to add complementary homopolymer tails to vector and insert DNA for construction of recombinant DNA *in vitro*.
T 6137	**Trioxsalen** (TMP), Minimum 98% (HPLC)	Photochemical crosslinker of DNA has been used as a probe for nucleic acid structure and function. Trioxsalen has also been used to crosslink DNA onto mica surfaces.

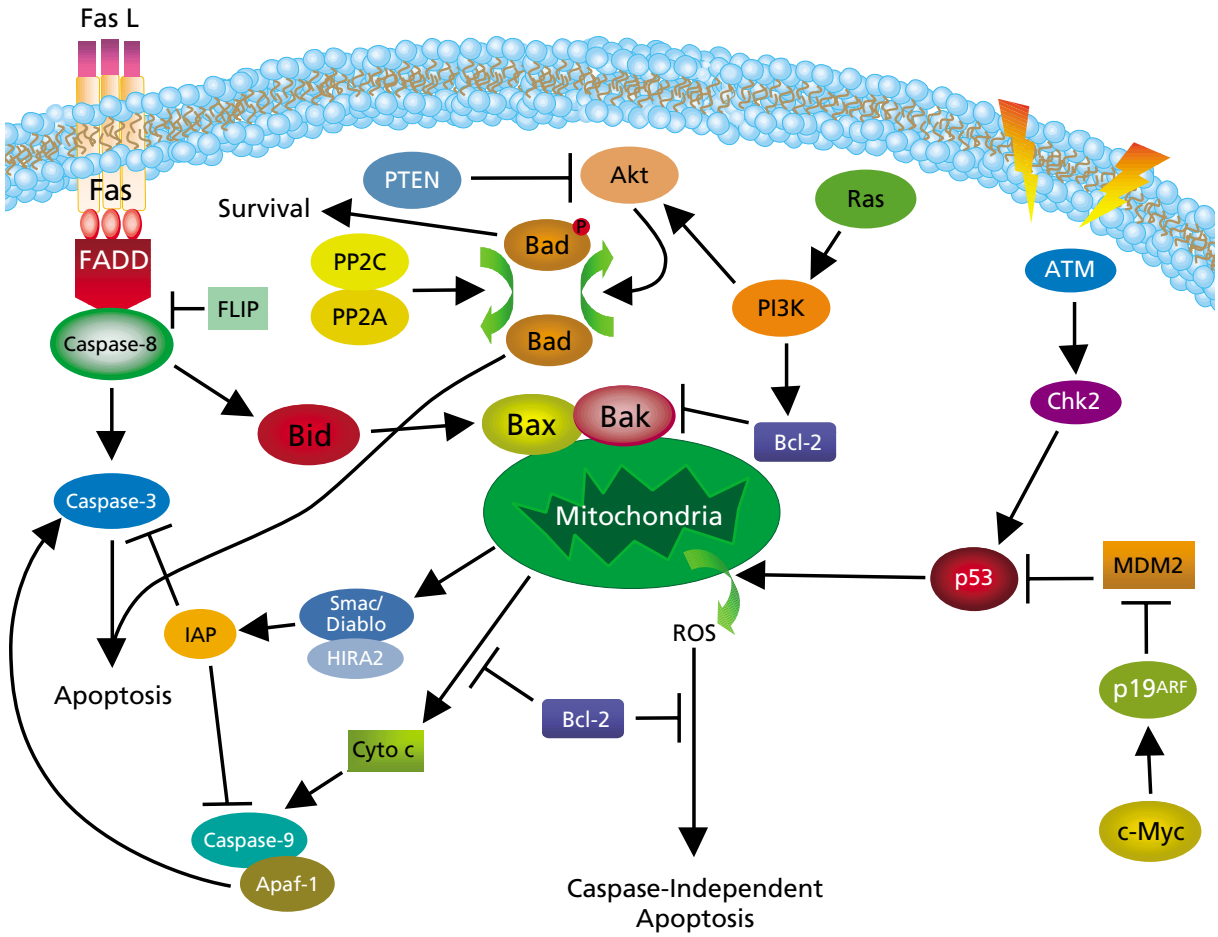
Apoptosis Inducers

Apoptosis Inducers		
	N-Acetyl-L-cysteine (LNAC; NAC)	Antioxidant and mucolytic agent. Reported to prevent apoptosis in neuronal cells but to induce apoptosis in smooth muscle cells. Inhibits HIV replication. May serve as a substrate for microsomal glutathione transferase.
A 7250	Minimum 99% (TLC)	
A 9165	Cell Culture Tested	
A 7191	**N-Acetyl-D-sphingosine** **semisynthetic** (C_2 ceramide; Acetyl ceramide) Approx. 98% (TLC)	Cell-permeable, biologically active ceramide. Induces differentiation and apoptosis in cells and has been shown to activate protein phosphatases.
	Actinomycin D **From *Streptomyces sp.***	An anti-neoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocks the movement of RNA polymerase that interferes with DNA-dependent RNA synthesis. Induces apoptosis. Potent anti-tumor agent.
A 1410	Approx. 98% (HPLC)	
A 9415	> 95%, Cell Culture Tested	
A 6885	**Adenosine 3′,5′-cyclic** **monophosphate** **sodium salt monohydrate** (3′,5′-Cyclic AMP sodium salt; cAMP-Na) Approx. 98% (HPLC)	Naturally-occurring activator of cyclic-AMP-dependent protein kinase (PKA). cAMP is an important second messenger that is linked in many systems to neurotransmitter- or hormone-induced receptor stimulation. The cAMP/PKA signaling pathway has been shown to inhibit cell proliferation, induce differentiation and lead to apoptosis.
P 8765	**Ammonium** **pyrrolidinedithiocarbamate** (PDTC; PDC; APDC) Approx. 99%	Prevents induction of nitric oxide synthetase by inhibiting translation of NOS mRNA; induces apoptosis in rat smooth muscle cells and inhibits apoptosis in leukemia HL-60 cells.
A 9789	**Anisomycin** **From *Streptomyces griseolus*** (2-[(4-Methoxyphenyl)methyl]-3,4-pyrrolidinediol 3-acetate; Flagecidin;) Approx. 97% (TLC)	Antibiotic that inhibits protein synthesis. Acts by inhibiting peptidyl transferase activity in eukaryote ribosomes. Reported to induce apoptosis in a variety of cells including promyelocytic leukemia cells, Jurkat cells, ventricular myocytes, and colon adenocarcinoma cells. Initiates intracellular signals and immediate early gene induction. Antiprotozoal agent.
A 8674	**Antimycin A** **From *Streptomyces sp.***	Inhibitor of electron transfer at complex III. Induces apoptosis.
	Arachidonic acid (AA; cis,cis,cis,cis-5,8,11,14-Eicosatetraenoic acid; Eicosa-5Z,8Z,11Z,14Z-tetraenoic acid)	An unsaturated ω6 fatty acid constituent of the phospholipids of cell membranes. AA and its metabolites play important roles in a variety of biological processes, including signal transduction, smooth muscle contraction, chemotaxis, cell proliferation and differentiation, and apoptosis. AA has been demonstrated to bind to the α subunit of G protein and inhibit the activity of Ras GTPase-activating proteins (GAPs).
A 9673	Approx. 99% (capillary GC) Oil	
A 8798	**Arachidonic acid sodium salt** Approx. 99% (capillary GC)	
A 0580	**Arachidonylethanolamide** (AEA; Arachidonic acid N-(hydroxyethyl)amide; Anandamide) Approx. 98% (TLC) Oil	An arachidonic acid derivative that appears to be an endogenous ligand for the cannabinoid receptor and for the vanilloid receptor, VR1. It Inhibits calcium currents in neuroblastomas and neurons. Shown to activate the MAP kinase signaling pathway. Inhibits proliferation and induces apoptosis of lymphocytes and human breast cancer cells.
B 1793	**Bafilomycin A1** **From *Streptomyces griseus*** Minimum 90% (HPLC)	A specific inhibitor of vacuolar type H^+-ATPase (V-ATPase) in animal cells, plant cells and microorganisms. Induces apoptosis in PC12 cells and the human pancreatic cancer cell line, Capan-1.



Apoptosis Inducers

Activation and Inhibition of Apoptosis



Biochemical studies have characterized apoptosis and several mechanisms have been identified in mammalian cells for induction of apoptosis. These mechanisms include factors that lead to perturbation of the mitochondria leading to leakage of cytochrome c or factors that directly activate members of the death receptor family. Fas is a member of the tumor necrosis factor (TNF) receptor superfamily, a family of transmembrane receptors that include neurotrophin receptor (p75NTR), TNF-R1, and a variety of other cell surface receptors. Fas Ligand (Fas L) transmits signals to Fas on a target cell by inducing trimerization of Fas. Activation of Fas causes the recruitment of Fas-associated protein with death domain (FADD) via interactions between the death domain of Fas and FADD and is followed by pro-caspase-8 binding to FADD via interactions between the death effector domains (DED) of FADD and pro-caspase-8 leading to the activation of caspase-8. Activation of caspase-8 leads to the activation of other caspases, in effect beginning a caspase cascade that ultimately leads to apoptosis. Caspase-8 activation can also activate Bid, leading to activation of the apoptotic program. Fas-induced apoptosis can be effectively blocked at several stages by either FLICE-inhibitory protein (FLIP), by Bcl-2, or by the cytokine response modifier A (CrmA). In addition, activation of caspase 3 by caspase 9 can be blocked by inhibitor of apoptosis proteins (IAPs).

Moreover, the protein kinase, Akt, can be activated by various growth factors and its activity can be blocked by PTEN. Akt functions to promote cell survival through two distinct pathways: Akt inhibits apoptosis by phosphorylating the Bcl-2 family member Bad, which then interacts with 14-3-3 and dissociates from Bcl-X_L allowing for cell survival alternatively, Akt activates IKK-α that ultimately leads to NF-κB activation and cell survival. Proapoptotic Bcl-2 family members, such as Bax and Bak can promote mitochondrial permeability, while Bcl-2 can inhibit their effects. Upon mitochondrial permeability, apoptogenic factors are released from the mitochondrial inter-membrane space and leak into the cytosol. One factor is cytochrome c, induces the liberation of protease activators (caspases) that ultimately lead to apoptosis through nuclear damage (DNA fragmentation, DNA mutations). In addition, Smac/Diablo is released and can block IAP inhibition of capsase activity. Mitochondrial permeability is also related to the increased generation of reactive oxygen species (ROS), which plays a role in the degradation phase of apoptosis (i.e. plasma membrane alterations).

Bcl-2 Family Proteins

Proteins		
B 1682	**Bad, mouse** Recombinant, expressed in *Escherichia coli* Minimum 70% (SDS-PAGE) Solution	A pro-apoptotic factor and a member of the Bcl-2 family. Cytokine-induced phosphorylation of Bad results in it being sequested in the cytosol. In the absence of cytokines such as interleukin 3 (IL-3), Bad dephosphorylation occurs, causing the release of cytochrome c from the mitochondria.
B 1182	**Bcl-2, human** Recombinant, expressed in *Escherichia coli* Minimum 90% (SDS-PAGE) Solution	The Bcl-2 family of proteins serve as critical regulators of pathways involved in apoptosis, acting either to inhibit or promote cell death. Bcl-2 has the ability to homodimerize or heterodimerize with death promoting Bcl-2 family members. Its anti-apoptotic activity may be related to its phosphorylation state and could be related to prevention of pore formation in the mitochondrial membrane.
B 1059 NEW	**Bcl-w (Minus C-Terminus), human** Recombinant, expressed in *Escherichia coli* >95% (SDS-PAGE) Solution	Like Bcl-2 and Bcl-x, the Bcl-w protein promotes cell survival, in contrast to other close homologues, Bax and Bak, which facilitate cell death. Bcl-w is an anti-apoptotic member of the Bcl-2 family that prevents release of cytochrome c from the mitochondrial inter-membrane space into the cytosol. It is required for normal sperm maturation. Natural Bcl-w contains a C-terminal mitochondrial targeting sequence. Recombinant Bcl-w lacks the mitochondrial targeting sequence but maintains the ability to neutralize pro-apoptotic Bcl-2 family members.
B 0934 NEW	**Bcl-x_L (Minus C-Terminus), human** Recombinant, expressed in *Escherichia coli* Approx. 95% (SDS-PAGE) Solution	When stably transfected into an interleukin 3 (IL-3)-dependent cell line, Bcl-x_L inhibits cell death upon growth factor withdrawal at least as well as Bcl-2. Natural Bcl-x contains a C-terminal mitochondrial targeting sequence. Recombinant Bcl-x lacks the mitochondrial targeting sequence but maintains the ability to neutralize pro-apoptotic Bcl-2 family members.
B 8056	**Bcl-x_L (Minus C-Terminus), mouse** Recombinant, expressed in *Escherichia coli* Minimum 95% (SDS-PAGE) Solution	
B 8181	**BID, human** Recombinant, expressed in *Escherichia coli* Minimum 95% (SDS-PAGE) Solution	Regulates outer mitochondrial membrane permeability, a pro-apoptotic protein that causes release of cytochrome c from the mitochondrial intermembrane space to the cytosol.
B 8306	**BID, mouse** Recombinant, expressed in *Escherichia coli* Minimum 95% (SDS-PAGE) Solution	
C 4608	**BID, Caspase-8-cleaved, human** Recombinant, expressed in *Escherichia coli* Minimum 95% (SDS-PAGE) Solution	Caspase-8-cleaved BID relocates from the cytosol to the outer mitochondrial membrane where it interacts with Bak and alters mitochondrial membrane permeability. BID is cleaved with caspase-8 to generate an N-terminal fragment (7 kDa) and the C-terminal fragment (15 kDa). On size exclusion chromatography cleaved BID elutes as a single band corresponding to 27 kDa indicating that the two fragments remain associated.
C 4733	**BID, Caspase-8-cleaved, mouse** Recombinant, expressed in *Escherichia coli* Minimum 95% (SDS-PAGE) Solution	
Antibodies		
B 0559 NEW	**Monoclonal Anti-Bad** Clone 64130, Produced in rat	Species reactivity: mouse Purified immunoglobulin
B 0684 NEW	**Anti-Bad** Produced in rabbit	Species reactivity: human, mouse Affinity isolated antibody
B 5679	**Anti-phospho-Bad (pSer^{112})** Produced in sheep	Species reactivity: mouse, human Affinity isolated antibody
B 5804	**Anti-phospho-Bad (pSer^{136})** Produced in sheep	Species reactivity: mouse, human Affinity isolated antibody
B 5897	**Anti-Bak** Produced in rabbit	Species reactivity: human IgG fraction of antiserum
B 9054	**Monoclonal Anti-Bax** Clone 5B7, Produced in mouse	Species reactivity: mouse Does not react with human, rat Purified immunoglobulin
B 8429	**Monoclonal Anti-Bax** Clone 6A7, Produced in mouse	Species reactivity: human, rat, mouse Purified immunoglobulin