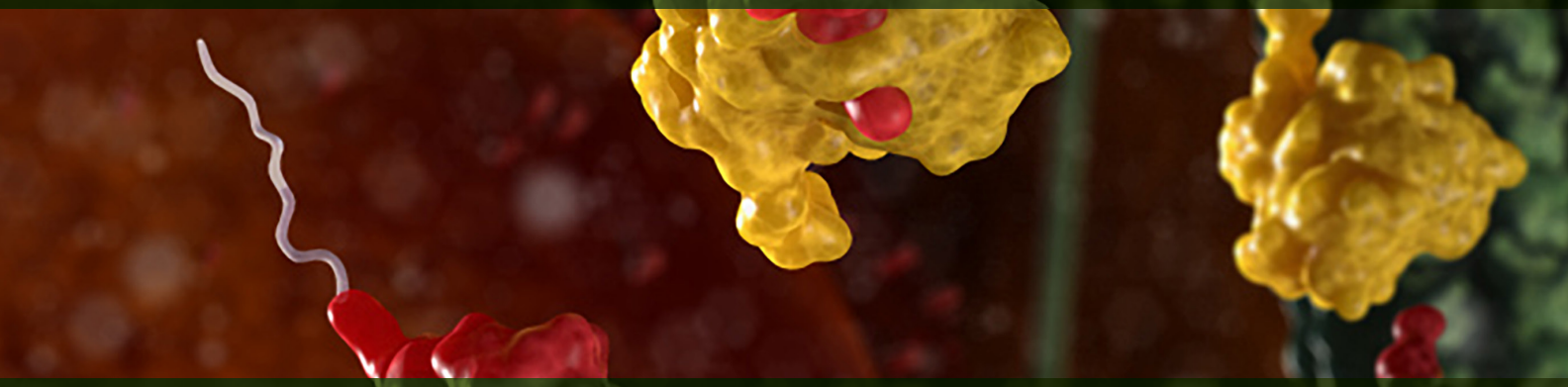
The Researcher's Guide to
Mechanisms of Cell Death



Cell Signaling TECHNOLOGY®

The Three Major Modes of Cell Death

Cell death is the process by which living cells cease to function and it can occur in many different ways for different reasons. Cells can die either during development, as a result of cellular stress and metabolic disruption, from pathogenic invasion, or due to physiological tissue damage.

Decades of research have allowed for the detailed characterization of the myriad ways that cells can self-destruct. Classically, cell death is categorized into three different types, based on morphological changes, triggers, and the biochemical pathways involved.

Apoptosis, or Type I cell death, is a tightly regulated form of programmed cell death (PCD) that triggers cells to self-destruct without any external influence. It is an essential part of life, particularly for multicellular organisms that must control the growth, development, and turnover of cells in order to maintain homeostasis. Apoptosis is critical for proper embryonic development, and a classic example of this process can be observed when cells between the digits on a hand apoptose in order to separate the fingers.

Autophagy, or Type II cell death, is also often categorized as a type of programmed cell death. However, over time it has become clear that it can promote cell survival as well as cell death, depending upon the circumstance. Autophagy is a process by which cellular organelles and other contents are devoured by lysosomes to clear away unnecessary or dysfunctional components. This critical mechanism allows for the systematic degradation and recycling of cellular materials. While autophagy is an important cellular mechanism, this guide will focus on other mechanisms of cell death, including apoptosis and regulated necrotic pathways.

Necrosis, or Type III cell death, has historically been thought of as a process that occurs accidentally due to extreme external physiological stress. In recent years, researchers have shed light on specific regulated molecular mechanisms that can be triggered when a cell is under stress and alternative methods of cell death are not available. This guide will describe some of these regulated necrotic cell death pathways, including **necroptosis**, **pyroptosis**, **ferroptosis**, and **NETosis**.

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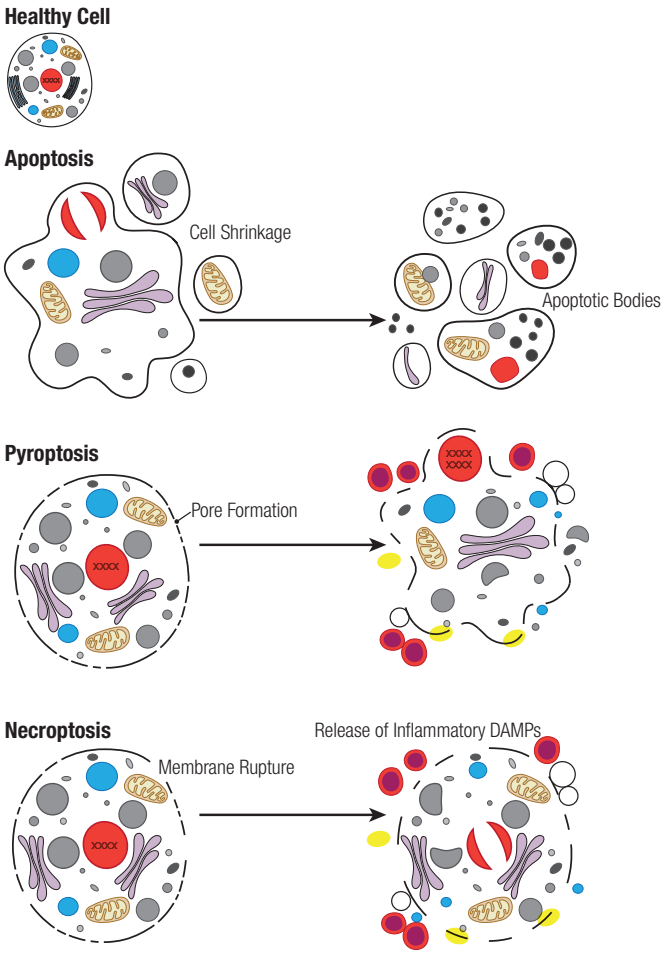
Differences in Cell Death Morphologies

One of the earliest and often-used methods of classifying different types of cell death is to identify the observable morphological differences between them. An apoptotic cell is morphologically characterized by cytoplasmic shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation. During apoptosis, membrane blebbing, or the malformation of the plasma membrane, can also lead to the formation of small vesicles, termed apoptotic bodies, around the edge of the dying cell. It is important to note that the cell membrane remains intact during apoptosis. However, phosphatidylserine (PS) lipids within the membrane, which normally face the cytosol, flip to become exposed to the extracellular space. Their presence acts as a signal for neighboring phagocytes to remove the dead cell.

Its ability to bind to PS lipids on the cell membrane has caused Annexin V to be used traditionally as a marker of early apoptosis in combination with reagents that confirm the integrity of the cell membrane, such as propidium iodide. Whether the cell membrane remains intact or not is critical information to differentiate apoptosis from necrotic cell death pathways. ***However, the presence of Annexin V staining alone, indicating PS exposure, is not enough to differentiate between types of cell death.***



In contrast, necrosis is often characterized by breakage of the cell membrane after cellular swelling and membrane blebbing, which leads to leakage of the cell contents that often induces inflammation of the affected tissue. This rupture may be caused by sudden overwhelming physiological forces applied to the cell or, alternatively, it may be the result of externally triggered and internally regulated cellular programming. Vacuoles and lysosomes are notably absent during necrotic cell death processes.

Within the category of necrotic cell death, regulated necrotic processes can display distinctive morphological features as well. During necroptosis, different stages of cell breakdown can be observed, including the swelling of organelles, rupture of the cell membrane, and eventually, the disintegration of the cytoplasm and nucleus. Cells that undergo pyroptosis display characteristics such as cell swelling, membrane blebbing, DNA fragmentation, and eventual cell lysis. However, the nucleus often remains intact, which differs from the nuclear destruction that can be observed during apoptosis and necroptosis. Ferroptosis results neither in the chromatin condensation observed during apoptosis nor in the loss of plasma membrane integrity that occurs during necrosis. Rather, it is characterized by the presence of condensed mitochondria, reduction in mitochondrial cristae, and increased membrane densities.

Summary of the different morphologies, mechanisms, and outcomes of the three forms of cell death (apoptosis, pyroptosis, and necroptosis)

	Characteristics	Apoptosis	Pyroptosis	Necroptosis
Morphology	Cell lysis	×	✔	✔
	Cell swelling	×	✔	✔
	Pore formation	×	✔	✔
	Membrane blebbing	✔	✔	×
	DNA fragmentation	✔	✔	✔
	Nucleus intact	×	✔	×
Mechanism	Caspase-1 activation	×	✔	×
	Caspase-3 activation	✔	✔	×
	GSDMD activation	×	✔	×
	RIP3 activation	×	×	✔
	MLKL activation	×	×	✔
Outcome	Inflammation	×	✔	✔
	Programmed cell death	✔	✔	✔

Apoptosis

Apoptosis is a tightly controlled pattern of programmed cell death characterized by distinct morphological changes along with the activation of specific caspases and mitochondrial control pathways. It can be triggered via intrinsic or extrinsic pathways. The intrinsic pathway can be activated by cell stress, DNA damage, developmental cues, or withdrawal of survival factors. The extrinsic pathway is triggered via the detection of extracellular death signals from other cells.

The intrinsic apoptotic pathway involves many conserved signaling proteins and depends upon the integrity of the mitochondrial membrane. This process is tightly regulated by a balance between the activity of proteins in the Bcl-2 family, which consists of pro-apoptotic and anti-apoptotic members. Apoptosis is triggered when pro-apoptotic family members with a BH3 domain, such as BAD, BID, and BIM, are activated during cellular stress via changes in expression or through post-translational modifications. Then pro-apoptotic proteins, BAX and BAK, induce changes in the mitochondrial outer membrane permeability (MOMP), leading to the release of cytochrome c from the organelle's intermembrane space. Free cytochrome c then forms a complex with Apaf-1, called the apoptosome, that activates caspase-9 and triggers a cascade of apoptotic events.

Anti-apoptotic family members, such as Bcl-2, Bcl-XL, and MCL-1, can bind to and inhibit pro-apoptotic proteins to prevent apoptosis. These proteins are often active in cancer cells and are therefore attractive targets for the development of cancer therapeutics. Translational research has focused on targeting these proto-oncogenic proteins in different ways; one strategy has been to design compounds to mimic BH3-containing pro-apoptotic proteins that induce apoptosis by competing with the interaction between pro- and anti-apoptotic family members.

Both the intrinsic and extrinsic apoptotic pathways are ultimately dependent upon the protease activity of specific members of the caspase family. These caspases fall into two main categories. The "initiator" caspases-2, -8, -9, -10, and -12 are coupled to upstream, pro-apoptotic signals and act by cleaving the downstream "executioner" caspases-3, -6, and -7, which modify proteins ultimately responsible for the disassembly of the cell. Cleavage-specific antibodies targeting these caspases or their substrates serve as important tools to identify cell death. For example, targets such as PARP and lamin A/C, are cleaved by executioner caspases and are useful markers of apoptosis. Additionally, proteins in the Inhibitors of Apoptosis (IAP) family, such as XIAP, block the activity of different caspases to prevent apoptosis, and their expression is an indicator of enhanced cell survival.

Caspases can also be activated through the extrinsic pathway upon activation from extracellular ligands that trigger cell surface death receptors. The death receptors consist of members of the TNFR family and their associated ligands include TNF-α, FasL, TRAIL, and TWEAK. Receptor activation leads to the formation of a Death Inducing Signaling Complex (DISC) that activates a pro-caspase. Members of the complex include adaptor proteins, FADD and TRADD, that recruit and activate the initiator caspase-8 and the executioner caspase-3. Interestingly, 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, FLIP, and XIAP.

KEY PRODUCTS FOR STUDYING APOPTOSIS

Annexin V-FITC Early Apoptosis Detection Kit #6592
Annexin V binds to phosphatidylserine lipids that can become exposed in the cell membrane and, when used in combination with propidium iodide, enables researchers to identify early apoptotic cells within a cell population.

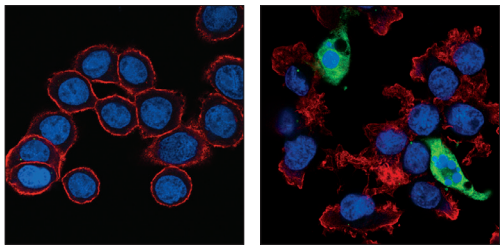
Bcl-xL (54H6) Rabbit mAb #2764
Mitochondrial expressed Bcl-xL inhibits apoptosis. Anti-apoptotic Bcl-2 family members are often overexpressed in cancer cells and are therefore attractive targets for the development of cancer therapeutics.

Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664
Caspase 3 is an executioner protein that is responsible for the disassembly of the cell. Cleavage specific antibodies targeting these caspases (or their substrates) are important tools to identify cell death.

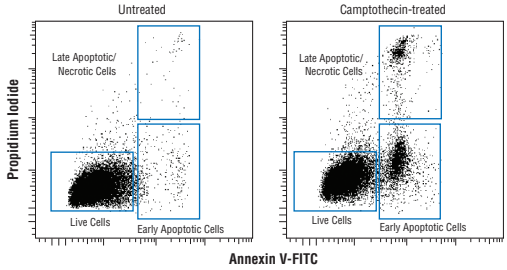
Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb #5625
PARP is commonly cleaved by executioner caspases and is a marker for apoptosis.

Cytochrome c (6H2.B4) Mouse mAb #12963
The release of cytochrome c from mitochondria is a hallmark of intrinsic apoptosis.

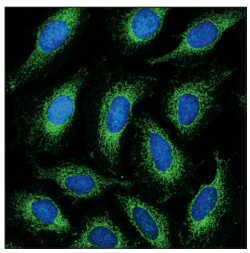
Mitochondrial Membrane Potential Assay Kit (II) #13296
Apoptosis progresses when changes in a cell's mitochondrial outer membrane permeability (MOMP) occur. Depolarized mitochondria in apoptotic cells show reduced fluorescent TMRE accumulation.



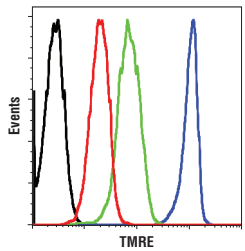
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664: Confocal IF images of HT-29 cells, untreated (left) or Staurosporine #9953 treated (right) labeled with #9664 (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin #8953 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



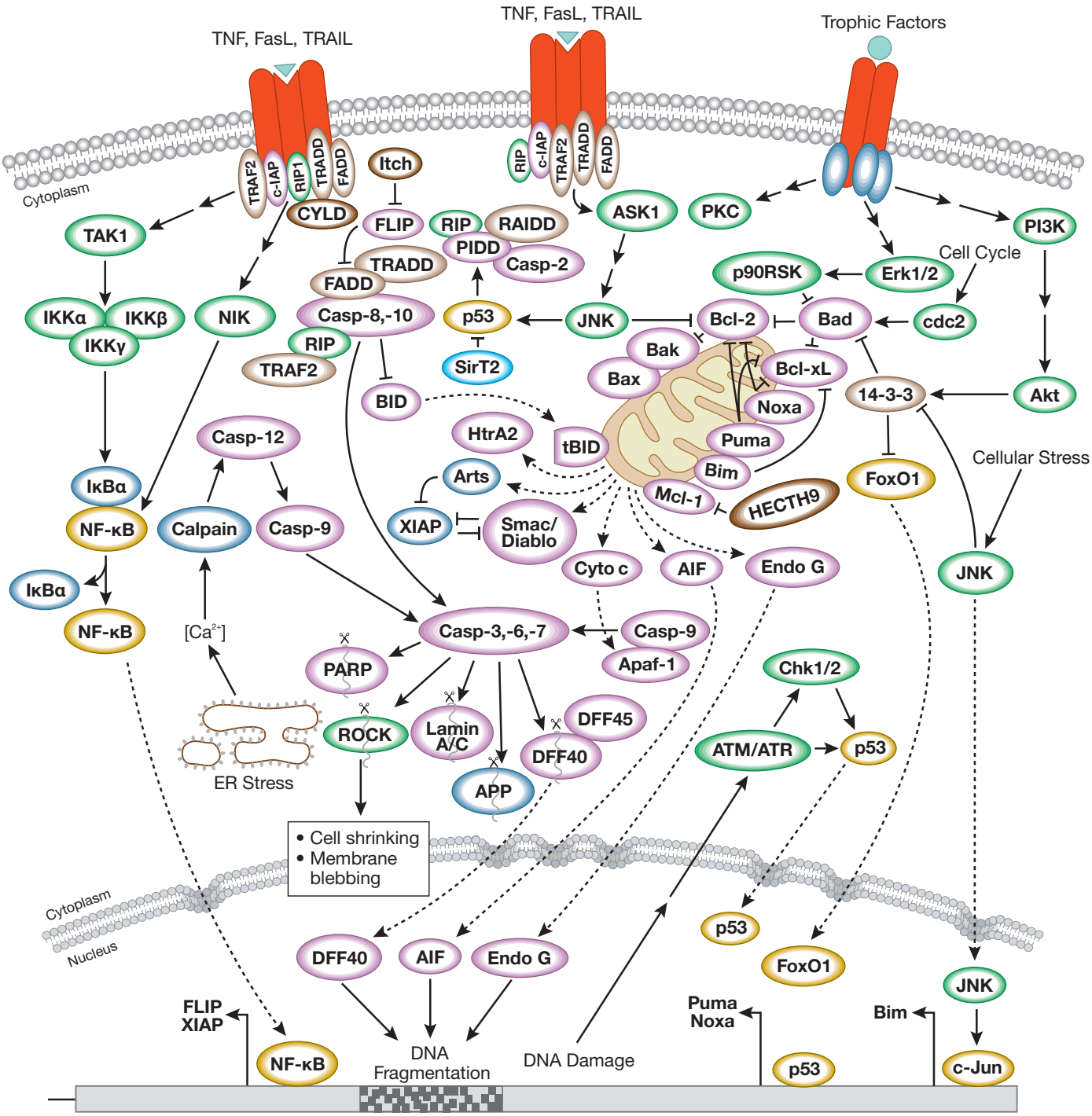
Annexin V-FITC Early Apoptosis Detection Kit #6592: Flow cytometric analysis of Jurkat cells untreated (left) or treated with camptothecin (10 µM, 4 hr; right) using #6592.



Bcl-xL (54H6) Rabbit mAb #2764: Confocal IF analysis of HeLa cells using #2764 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



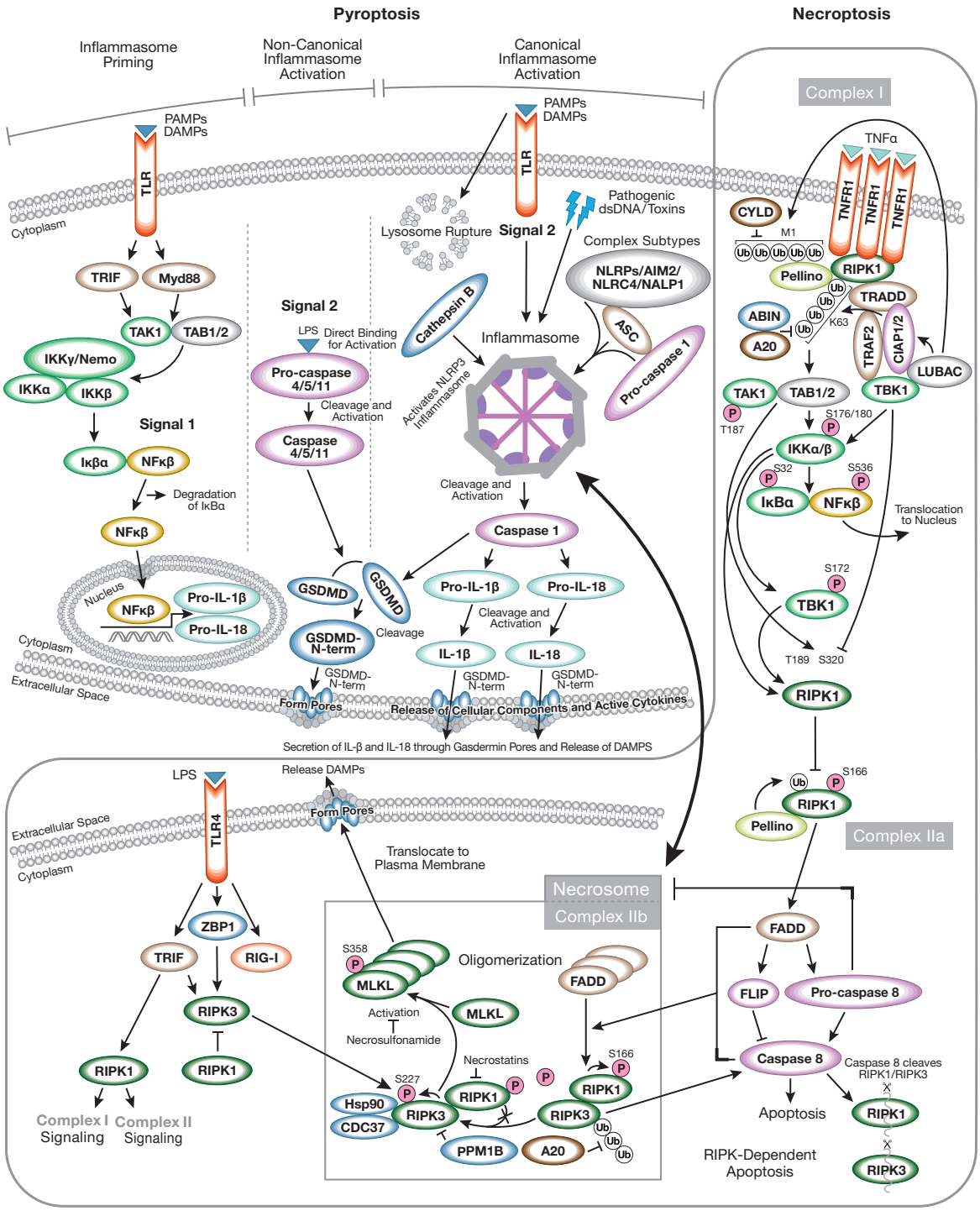
Mitochondrial Membrane Potential Assay Kit (II) #13296: Flow cytometric analysis of Jurkat cells, unlabeled (black) or labeled with 200 nM TMRE and treated with 0 µM CCCP (blue), 3.2 µM CCCP (green), or 80 µM CCCP (red).


TNF, FasL, TRAIL
TNF, FasL, TRAIL
Trophic Factors
Cytoplasm
TRAF2
c-IAP
RIP1
TRADD
FADD
CYLD
Itch
RIP
c-IAP
TRAF2
TRADD
FADD
TAK1
FLIP
RIP
RAIDD
PIDD
Casp-2
TRADD
FADD
ASK1
PKC
PI3K
Cell Cycle
p90RSK
Erk1/2
cdc2
IKKα
IKKβ
IKKγ
NIK
Casp-8,-10
RIP
TRAF2
p53
SirT2
JNK
Bcl-2
Bad
Bak
Bax
Bcl-xL
Noxa
14-3-3
Akt
BID
HtrA2
tBID
Puma
Bim
Mcl-1
HECTH9
FoxO1
Cellular Stress
Casp-12
IκBα
NF-κB
Calpain
Casp-9
Arts
XIAP
Smac/
Diablo
Cyto c
AIF
Endo G
JNK
IκBα
NF-κB
[Ca2+]
Casp-3,-6,-7
Casp-9
Apaf-1
PARP
Chk1/2
ROCK
Lamin
A/C
DFF45
DFF40
ATM/ATR
p53
ER Stress
APP
• Cell shrinking
• Membrane
blebbing
Cytoplasm
Nucleus
p53
DFF40
AIF
Endo G
FoxO1
JNK
FLIP
XIAP
NF-κB
DNA
Fragmentation
DNA Damage
Puma
Noxa
p53
Bim
c-Jun

Necrosis

Necrosis has been classically defined as unprogrammed cell death that occurs in response to overwhelming chemical or physical insult. External forces that may lead to this accidental cell death include extreme physical temperature, pressure, chemical stress, or osmotic shock. Rupture of the cell, which is characteristic of necrosis, leads to leakage of the cellular contents into the extracellular space.

This causes the release of biomolecules, called damage-associated molecular patterns (DAMPs), that can be recognized by immune cells and trigger an inflammatory response. However, recent studies have uncovered several regulated pathways that can also trigger necrotic cell death. These programmed necrosis pathways include **necroptosis**, **pyroptosis**, and **ferroptosis**.



Necroptosis

Necroptosis is a process of cellular self-destruction that is activated via extracellular signals (death receptor-ligand binding) or intracellular cues (microbial nucleic acids) when apoptosis is otherwise prevented. Necroptosis is highly immunogenic in nature and is useful as a host defense mechanism against pathogens. It is also activated in inflammatory diseases, neurodegeneration, and cancer.

Necroptosis is a distinct form of programmed necrotic cell death that progresses independently of caspase activity. Instead, it requires the RIPK3-dependent phosphorylation of MLKL (Ser358 in human, Ser345 in mouse). This phosphorylation event allows MLKL to generate a pore complex in the plasma membrane leading to the secretion of DAMPs, cell swelling, and membrane rupture.

In the canonical pathway, RIPK3 is activated via phosphorylation at Ser227 (Thr231/Ser232 in mouse) after forming a complex with RIPK1. RIPK1 activity can be monitored by examining autophosphorylation sites, such as Ser166 and Ser14, using phosphorylation-specific antibodies. It is important to note that RIPK1 is highly regulated and plays a role in complexes that initiate NF-κB signaling and survival, apoptosis, or necroptosis. Thus, analyzing the phosphorylation states of the key proteins RIPK1, RIPK3, and MLKL is an important step in identifying necroptosis. Alternate triggers of this pathway include pathogen activation of TRIF and ZBP-1 that subsequently interact with and activate RIPK3.

Necroptosis can be inhibited in various ways, including via the use of small molecules called necrostatins that directly inhibit RIPK1 activity. Additionally, active caspase-8 can inhibit necroptosis through cleavage of RIPK1 and RIPK3. In this way, the caspase-driven apoptotic pathway inhibits necroptosis. Conversely, activation of the protein FLIP, which is a catalytically inactive homolog of caspase-8, can prevent RIPK1 cleavage and drive necroptosis.

KEY PRODUCTS FOR STUDYING NECROPTOSIS

RIP (E8S7U) XP® Rabbit mAb #73271
Necroptosis is initiated by the formation of a complex called the necrosome, which includes RIP kinase (RIPK1) and RIPK3.

Phospho-RIP (Ser166) (D1L3S) Rabbit mAb #65746
Autophosphorylation of RIPK1 at Ser166 is induced by the necropsosis signals Z-VAD, SM164, and TNF; and is inhibited by necrostatins

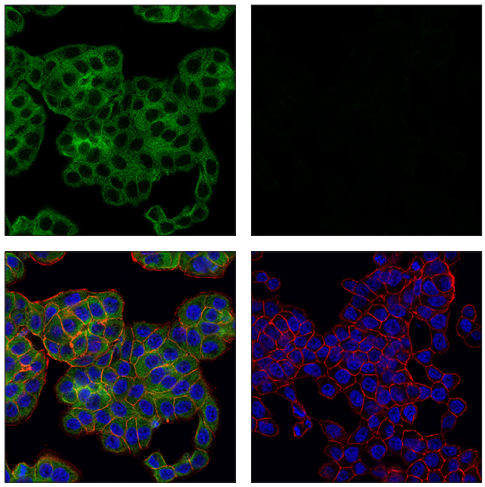
Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654
RIPK3 phosphorylation at Ser227, which is induced by interactions with RHIM domain proteins like RIPK1, is required for necroptosis.

Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb #37333
MLKL phosphorylation, which is induced by RIPK3, triggers pore formation and is required for necroptosis.

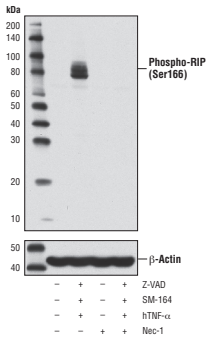
ANTIBODY SAMPLER KITS FOR STUDYING NECROPTOSIS

Necroptosis Antibody Sampler Kit #98110
RIP (D94C12) XP® Rabbit mAb #3493, Phospho-RIP (Ser166) (D1L3S) Rabbit mAb #65746, MLKL (D2I6N) Rabbit mAb #14993, Phospho-MLKL (Ser358) (D6H3V) Rabbit mAb #91689, RIP3 (E1Z1D) Rabbit mAb #13526, Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654, Anti-rabbit IgG, HRP-linked Antibody #7074

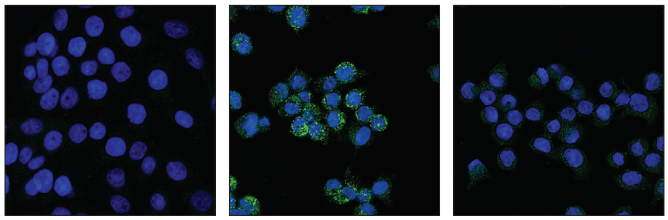
Mouse Reactive Necroptosis Antibody Sampler Kit #47928
Phospho-RIP (Ser166) (E7G6O) Rabbit mAb #53286, RIP (D94C12) XP® Rabbit mAb #3493, Phospho-RIP3 (Thr231/Ser232) (E7S1R) Rabbit mAb #91702, RIP3 (D8J3L) Rabbit mAb #15828, Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb #37333, MLKL (D6W1K) Rabbit mAb (Mouse Specific) #37705, Anti-rabbit IgG, HRP-linked Antibody #7074



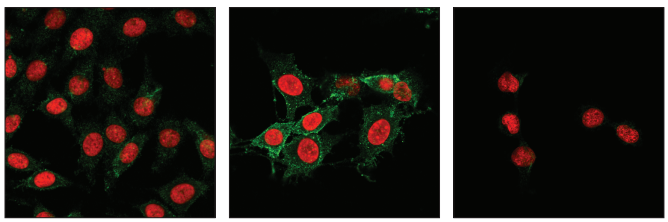
RIP (E8S7U) XP® Rabbit mAb #73271: Confocal IF analysis of HT-29 cells, either wild-type (left, positive) or RIPK1 knockout (-/-) (right, negative), using #73271 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).



Phospho-RIP (Ser166) (D1L3S) Rabbit mAb #65746: WB analysis of HT-29 cells, untreated (-) or treated with combinations of the following treatments as indicated: Z-VAD (20 µM, added 30 min prior to other compounds; +), human TNF-α (hTNF-α, 20 ng/ml, 7 hr; +), SM-164 (100 nM, 7 hr; +), and necrostatin-1 (Nec-1, 50 µM, 7 hr; +), using #65746 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).



Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654: Confocal IF analysis of HT-29 cells, untreated (left), pre-treated with Z-VAD (20 µM, 30 min) followed by treatment with SM-164 (100 nM) and Human Tumor Necrosis Factor-α (hTNF-α) #8902 (20 ng/mL, 6 hr; center), or pre-treated with Z-VAD followed by treatment with SM-164 and hTNF-α and post-processed with λ-phosphatase (right), using #93654 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb #37333: Confocal IF analysis of L-929 cells, untreated (left), pre-treated with Z-VAD (20 µM, 30 min) followed by treatment with SM-164 (100 nM) and Mouse Tumor Necrosis Factor-α (mTNF-α) #5178 (20 ng/mL, 2.5 hr; center) and then post-processed with λ-phosphatase (right), using #37333 (green). Red = Propidium Iodide (PI)/RNase Staining Solution #4087 (fluorescent DNA dye).

Pyroptosis

Pyroptosis is a type of cell death that is activated upon intracellular infections from bacteria, viruses, fungi, and protozoa in the presence of pathogen-associated molecular patterns (PAMPs) or cell-derived DAMPs. It is typically induced in cells of the innate immune system, such as monocytes, marcrophages, and dendritic cells.

Pyroptosis is characterized by the N-terminal cleavage of gasdermin D (GSDMD), which results in its oligomerization to form a lytic pore in the plasma membrane. This cleavage process relies on the activity of inflammatory caspases 1, 4, 5, and 11. Monitoring cleavage of GSDMD and its related family members, as well these inflammatory caspases, are key to studying pyroptosis.

The canonical pathway is often described as occurring through a two-step process. In the first "priming signal" step, NF-κB is activated to induce the expression of a number of proteins that will become part of a complex called the inflammasome. Inflammasomes typically consist of a cytosolic-pattern recognition receptor (PPR; such as NLRP3, or AIM2-like family members), an adaptor protein, such as ASC/TMS1, and pro-caspase-1. Analyzing the activation of the inflammasome via the detection of NLRP3 and visualization of ASC speckling is another useful technique to monitor pyroptosis.

In the second activation step, caspase-1 is proteolytically activated before cleaving gasdermin D and the cytokines, pro-IL1β and pro-IL18, creating their pro-inflammatory forms, IL1β and IL18, which are secreted from the dying cell. Visualizing the presence of these important cytokines can be achieved through the use of specific antibodies. Once GSDMD is cleaved by caspase-1 to form a membrane pore, cytokines are secreted, and the influx of water eventually leads to cell rupture.

Alternatively, pyroptosis can be induced via the non-canonical pathway by the intracellular detection of Gram-negative bacterial LPS, which activates caspase-4, -5 (human) and caspase-11 (mouse) to cleave GSDMD.

KEY PRODUCTS FOR STUDYING PYROPTOSIS

Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb #89332
Caspase-1 is proteolytically activated during canonical pyroptosis and is secreted out of the cell. Antibodies specific for cleaved caspase-1 can be used to monitor pyroptosis.

Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb #36425
The N-terminal fragment of gasdermin D, which is produced via cleavage by inflammatory caspases, can be used to monitor pyroptosis.

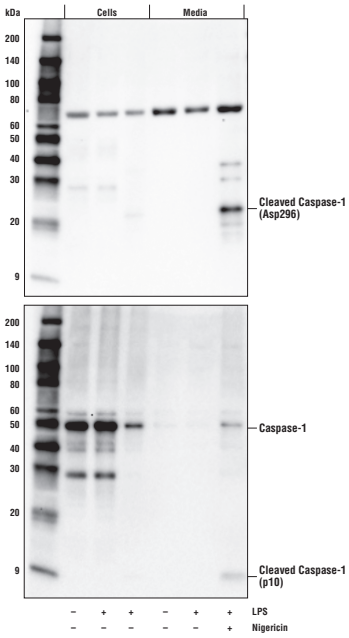
Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb #10137
Gasdermin D plays a critical role acting as a downstream effector of pyroptosis by oligomerizing to form a lytic pore in the plasma membrane.

Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb #83186
IL-1β is activated via cleavage by caspase-1 and secreted by cells undergoing pyroptosis.

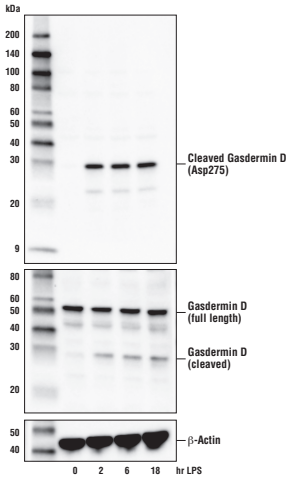
ANTIBODY SAMPLER KITS FOR STUDYING PYROPTOSIS

Pyroptosis Antibody Sampler Kit #43811
Gasdermin D (E8G3F) Rabbit mAb #97558, Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb #36425, Caspase-1 (D7F10) Rabbit mAb #3866, Cleaved Caspase-1 (Asp297) (D57A2) Rabbit mAb #4199, IL-1β (D3U3E) Rabbit mAb #12703, Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb #83186, Caspase-4 Antibody #4450, Caspase-5 (D3G4W) Rabbit mAb #46680, HMGB1 (D3E5) Rabbit mAb #6893, Anti-rabbit IgG, HRP-linked Antibody #7074

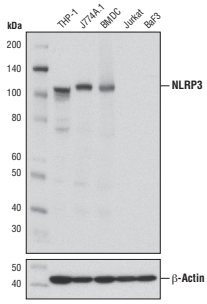
Mouse Reactive Pyroptosis Antibody Sampler Kit #98303
Gasdermin D (E9S1X) Rabbit mAb #39754, Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb #10137, IL-1β (D3H1Z) Rabbit mAb (Mouse Specific) #12507, Cleaved-IL-1β (Asp117) (E7V2A) Rabbit mAb (Mouse Specific) #63124, Caspase-1 (E2Z1C) Rabbit mAb #24232, Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb #89332, Caspase-11 (17D9) Rat mAb #14340, ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific) #67824, HMGB1 (D3E5) Rabbit mAb #6893, Anti-rabbit IgG, HRP-linked Antibody #7074



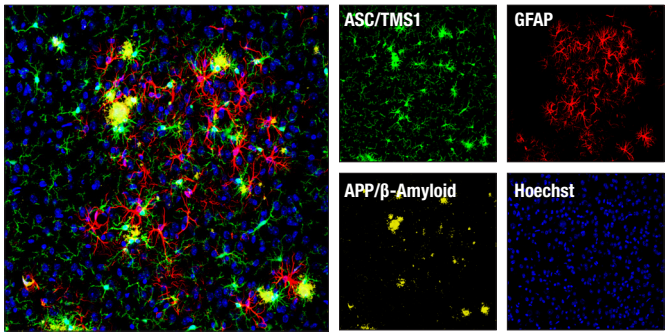
Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb #89332: WB analysis of cell extracts from the cells or media from mouse bone marrow-derived macrophages (mBMDM), untreated (-) or treated with Lipopolysaccharides (LPS) #14011 (50 ng/ml, 4 hr) followed by Nigericin (15 µM, 45 min) (+), using #89332 (upper), or Caspase-1 (E2Z1C) Rabbit mAb #24232 (lower).



Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb #36425: WB analysis of extracts from THP-1 cells, differentiated with TPA #4174 (50 ng/ml, overnight) and then treated with LPS #14011 (5 µg/ml, indicated times), using #36425 (upper), total Gasdermin D (L60) Antibody #93709 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).



NLRP3 (D4D8T) Rabbit mAb #15101: WB analysis of extracts from mouse bone marrow-derived dendritic cells (BMDC) and various cell lines using #15101 (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).



ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific) #67824: Confocal IF analysis of mouse Tg2576 brain which overexpresses mutant human APP695. Sections were first labeled with #67824 (green) and APP/β-Amyloid (NAB228) Mouse mAb #2450 (yellow). After blocking free secondary binding sites with Mouse (G3A1) mAb IgG1 Isotype Control #5415, sections were incubated with GFAP (GA5) Mouse mAb (Alexa Fluor® 647 Conjugate) #3657 (red). Nuclei were labeled with Hoechst 33342 #4082 (blue).

Ferroptosis

Ferroptosis is a recently described form of programmed cell death with distinct morphological and biochemical characteristics. It is an iron-dependent cell death that results in an increase in lipid peroxides. As such, it is largely regulated by pathways that contribute to iron homeostasis and oxidative stress. Iron homeostasis is controlled, in part, by ferratin, which is a large protein complex that is responsible for the transport of iron in its non-toxic form. Ferratin levels can be regulated by a selective autophagy process targeting ferratin, termed ferritinophagy. This pathway is mediated by a selective cargo receptor for ferratin called nuclear receptor coactivator 4 (NCOA4). Ferroptosis has been shown to be inhibited with the use of some common iron chelators, such as deferoamine.

Pathways regulating cellular defense against oxidative stress are critical to mitigate ferroptosis. Small-molecule lipophilic free radical scavengers, like ferrostatin-1 and liproxstatin-1, can inhibit ferroptosis. The glutathione pathway, in particular, has been identified as a key antioxidant defense pathway. A central player in this process is the metabolic protein glutathione peroxidase 4 (GPX4), which converts GSH into oxidized glutathione (GSSH), thus protecting cells against ferroptosis by limiting cytotoxic lipid peroxidation. The glutathione peroxidase pathway is further regulated by System Xc-, an amino acid antiporter consisting of a heterodimer of SLC7A11 and SLC3A2, which is critical for glutathione (GSH) synthesis. Importantly, one way to induce ferroptosis is to use the compound erastin that directly inhibits the function of System Xc-.

Regulation of genes involved in oxidative stress, including GPX4, are largely controlled by the transcription factor NRF2. This serves as a critical defense against ferroptosis. Under normal conditions, expression of NRF2 is inhibited through interaction with KEAP1, part of a ubiquitin E3 ligase complex that leads to NRF2 proteasomal degradation. Oxidative stress leads to conformation changes in KEAP1 that disrupts this interaction, resulting in stabilization of NRF2. This process is further regulated through the autophagy pathway in which the autophagy cargo receptor p62/SQSTM1 can competitively inhibit the KEAP1-NRF2 complex, leading to upregulation of NRF2.

Tools to monitor ferroptosis may involve multiple approaches including pharmacological sensitivity (such as iron chelators, antioxidants), changes in expression of targets (GPX4, SCL7A11, ferratin, NRF2, etc.), monitoring reactive oxygens and lipid peroxidation, and glutathione assays.

KEY PRODUCTS FOR STUDYING FERROPTOSIS

Cellular Glutathione Detection Assay Kit #13859
The presence of reduced glutathione is important for preventing cellular damage caused by reactive oxygen species. Depletion of glutathione is an indicator of the progression of cell death.

FTH1 (D1D4) Rabbit mAb #4393
Ferritin (FTH) is a ubiquitous and highly conserved protein that plays a major role in iron homeostasis by sequestering and storing iron.

GPX4 Antibody #52455
Glutathione peroxidase 4 (GPX4) protects cells against ferroptosis by limiting cytotoxic lipid peroxidation.

KEAP1 (D6B12) Rabbit mAb #8047
Regulation of the genes controlling oxidative stress serves as a critical defense against ferroptosis. Under normal conditions, expression of NRF2 is inhibited through interaction with KEAP1.

NCOA4 (E8H8Z) Rabbit mAb #66849
Nuclear receptor coactivator 4 (NCOA4) is a selective cargo receptor that mediates ferratin levels and is involved in the process of ferritinophagy.

NRF2 (D1Z9C) XP® Rabbit mAb #12721
Following oxidative or electrophilic stress, NRF2 is released from KEAP1, thereby allowing this transcriptional activator to translocate to the nucleus and regulate oxidative stress response genes.

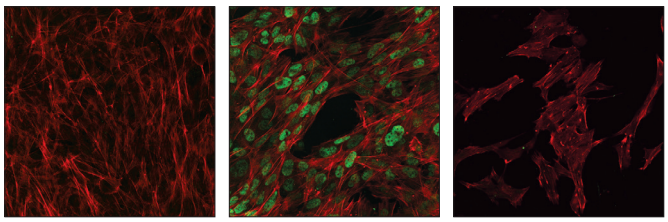
NRF2 (D9J1B) Rat mAb (IF Specific) #14596
This NRF2 antibody is specifically designed for use with immunofluorescence protocols to visualize the localization of NRF2 within cells.

xCT/SLC7A11 (D2M7A) Rabbit mAb #12691
xCT/SLC7A11 is part of the heterodimeric amino acid transport system x(c)(-) that regulates intracellular glutathione (GSH) levels, which is essential for cellular protection from oxidative stress.

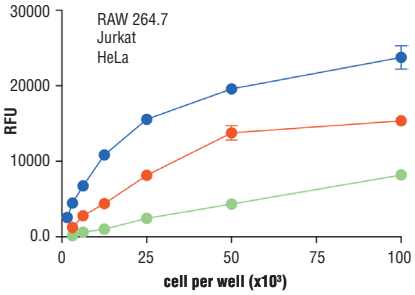
ANTIBODY SAMPLER KITS FOR STUDYING FERROPTOSIS

Ferroptosis Antibody Sampler Kit #29650
GPX4 Antibody #52455, NCOA4 (E8H8Z) Rabbit mAb #66849, KEAP1 (D6B12) Rabbit mAb #8047, NRF2 (D1Z9C) XP® Rabbit mAb #12721, 4F2hc/CD98 (D3F9D) XP® Rabbit mAb #47213, FTH1 (D1D4) Rabbit mAb #4393, xCT/SLC7A11 (D2M7A) Rabbit mAb #12691, DMT1/SLC11A2 (D3V8G) Rabbit mAb #15083, Anti-rabbit IgG, HRP-linked Antibody #7074

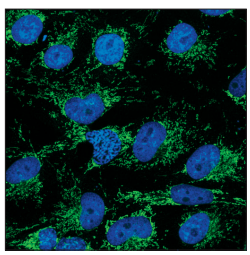
Redox Homeostasis and Signaling Antibody Sampler Kit #16815
GPX1 (C8C4) Rabbit mAb #3286, GPX4 Antibody #52455, Thioredoxin 1 (C63C6) Rabbit mAb #2429, Thioredoxin 2 (D1C9L) Rabbit mAb #14907, TRXR1 (D1T3D) Rabbit mAb #15140, TXNIP (D5F3E) Rabbit mAb #14715, Prdx1 (D5G12) Rabbit mAb #8499, Phospho-Prdx1 (Tyr194) (D1T9C) Rabbit mAb #14041, Anti-rabbit IgG, HRP-linked Antibody #7074



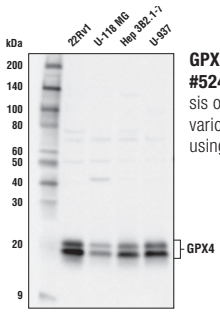
NRF2 (D9J1B) Rat mAb (IF Specific) #14596: Confocal IF analysis of MEF NRF2 wild-type cells, untreated (left) or treated with MG-132 #2194 (10 µM, 8 hr; center) and MEF NRF2 knock-out cells treated with MG-132 #2194 (10 µM, 8 hr; right), using #14596 (green pseudocolor). Actin filaments were labeled with Alexa Fluor® 488 Phalloidin #8878 (red pseudocolor).



Cellular Glutathione Detection Assay Kit #13859: RAW 264.7, HeLa, and Jurkat cells were seeded in 96-well plates at various cell densities. Cellular glutathione levels were determined using #13859 with live cells.



KEAP1 (D6B12) Rabbit mAb #8047: Confocal IF analysis of OVCAR8 cells using #8047 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



GPX4 Antibody #52455: WB analysis of extracts from various cell lines using #52455.

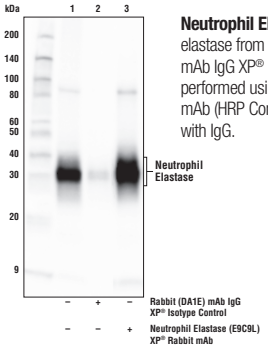
NETosis

NETosis is a unique form of regulated cell death that is characterized by membrane rupture and the extrusion of chromatin, histones, and granular and cytoplasmic components into a web-like structure called neutrophil extracellular traps (NETs). NETosis was identified as a response to bacterial infection and can be activated by lipopolysaccharides (LPS) as well as inflammatory pathway activators like phorbol 12-myristrate 13-acetate (PMA). NETosis can occur via multiple pathways, but several key players have emerged. The calcium dependent enzyme protein arginine deiminase 4 (PAD4) catalyzes hypercitrullination of histones that contributes to chromatin decondensation. In addition, release of neurophil elastase (ELANE) and myeloperoxidase (MPO) from cytoplasmic granules leads to disassembly of cytoskeletal structures and degradation of histones. Activation of NETosis may also occur following accumulation of reactive oxygen species (ROS). The pathological significance and potential therapeutic role of NETosis continues to be studied, but has been associated with host defense against pathogens as well as a number of disease states, including autoimmune diseases, thrombosis, cardiovascular diseases, and tumor progression.

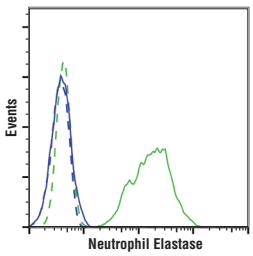
KEY PRODUCTS FOR STUDYING NETOSIS

Myeloperoxidase (E1E7I) XP® Rabbit mAb #14569
Release of neutrophil elastase and myeloperoxidase from cytoplasmic granules leads to disassembly of cytoskeletal structures and degradation of histones.

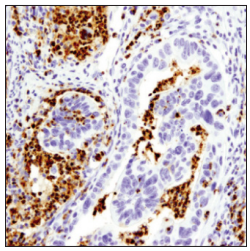
Neutrophil Elastase (E9C9L) XP® Rabbit mAb #89241
Neutrophils play a significant role in mediating the inflammatory response through the release of neutrophil elastase, which activates pro-inflammatory cytokines and degrades components of the extracellular matrix



Neutrophil Elastase (E9C9L) XP® Rabbit mAb #89241: IP of neutrophil elastase from THP-1 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is #89241. WB analysis was performed using #89241. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb (HRP Conjugate) #5127 was used for detection to avoid cross-reactivity with IgG.



Neutrophil Elastase (E9C9L) XP® Rabbit mAb #89241: Flow cytometric analysis of Jurkat cells (blue) and U-937 cells (green) using #89241 (solid lines) or a concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

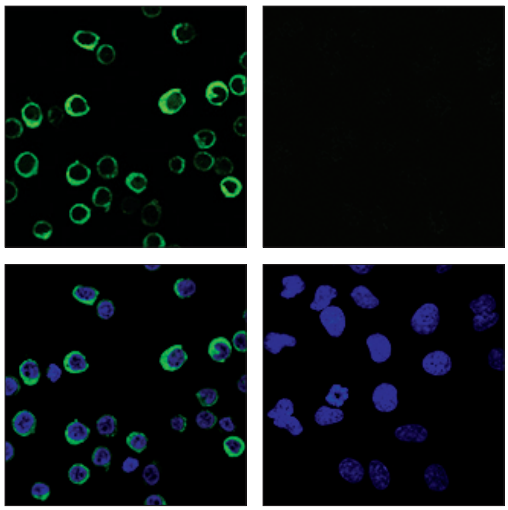


Myeloperoxidase (E1E7I) XP® Rabbit mAb #14659: IHC analysis of paraffin-embedded colon adenocarcinoma using #14659.

CELL DEATH PATHWAY CROSSTALK

Although different cell death pathways utilize distinct proteins and methods of cellular destruction, crosstalk between the pathways can occur. Both apoptosis and necroptosis can be driven by the activation of TNF receptors. Similarly, RIPK1 can be involved in the progression of either of these forms of cell death. Additionally, the molecular machinery between apoptosis and necroptosis has been shown to be linked in ways that make them mutually exclusive processes that regulate each other. Caspase-8, which is an "initiator" protein during apoptosis, not only cleaves proteins to drive apoptosis forward, but also cleaves RIPK1 and RIPK3 to prevent the progression of necroptosis. Importantly, while caspases are often thought of as markers of apoptosis, caspase-1, -4, and -5 are active during pyroptosis and play an important role in the innate immune system. Caspase-3 is also active during both apoptosis and pyroptosis. This enzyme can cleave gasdermin D to prevent pore formation and the progression of pyroptosis. Alternatively, recent evidence suggests that caspase-3 can also cleave gasdermin E to activate a pore complex, which may allow pyroptosis to progress with variable cleavage machinery.

The complex nature of each pathway along with what we know about crosstalk between these processes highlights how important it is to examine multiple readouts when identifying forms of cell death in experiments. Investigating more than one molecular pathway and cell death marker is imperative to obtain a clear picture of how cells die under different circumstances.

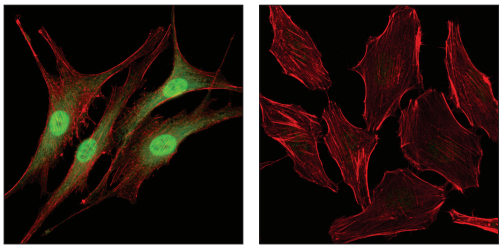


Myeloperoxidase (E1E7I) XP® Rabbit mAb #14569: Confocal IF analysis of HL-60 cells (left, positive) or HeLa cells (right, negative) using #14569 (green). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

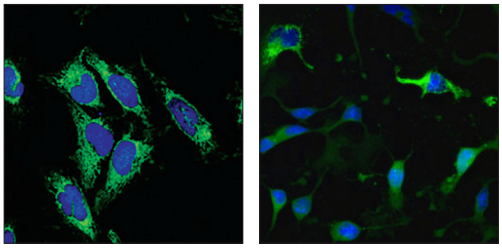
Cell Death and Disease

APOPTOSIS IN CANCER

The importance of characterizing different cell death pathways has become clearer as studies elucidate specific cell death mechanisms and identify targeted therapeutics. Loss of apoptosis has long been known to be a hallmark of cancer. Cancer cells evade apoptosis through overexpression of anti-apoptotic proteins, including Bcl-2 and IAP family members, as well as through the suppression of pro-apoptotic signals, such as p53. Small molecule therapies that restore function to mutant p53 or inhibit its interactions with oncogenic proteins have been shown to be effective against tumor cells. Drugs that target and inhibit IAPs, including mimetics of the protein SMAC, have shown promise for their ability to induce apoptosis. Additionally, therapeutic agents, including BH3 mimetics, that inhibit or prevent the expression of anti-apoptotic proteins, have been shown to be effective against lung cancer and lymphoma in clinical trials. These targets continue to be studied to develop new potential therapeutic treatments for cancer.



Livin (D61D1) XP® Rabbit mAb #5471: Confocal IF analysis of SK-MEL-28 cells (left) and HeLa cells (right) using #5471 (green). Actin filaments were labeled with DY-554 phalloidin (red).



Smac/Diablo (D5S3R) Rabbit mAb #15108: Confocal IF analysis of HeLa cells, untreated (left) or treated with Z-VAD (50 µM, 3 hr) and Staurosporine #9953 (1 µM, 3 hr; right), using #15108. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

NECROTIC CELL DEATH IN DISEASE

Forms of necrotic cell death, particularly the programmed processes, have been implicated in the pathology of many diseases, including inflammatory autoimmune diseases, fibrosis, and neurodegeneration-related diseases, such as Alzheimer's Disease (AD), multiple sclerosis (MS), and Amyotrophic Lateral Sclerosis (ALS). Necroptosis, in particular, has been shown to play a role in neurodegenerative diseases. Research from clinical trials has shown that treatment with Necrostatin-1, a RIPK1 inhibitor, improved cell viability in Alzheimer's and Parkinson's diseases. Studies have also identified inhibitors that directly target RIPK3 and MLKL.

Similarly, pyroptosis has been shown to be involved in neurodegenerative and autoimmune diseases, as well as acute tissue injury, such as ischemia-reperfusion damage. Currently, gasdermin D inhibitors are being investigated for their therapeutic potential to treat autoimmune and inflammatory diseases. Also, therapeutic strategies targeting IL-1β and its receptor have been developed for the treatment of arthritis and other inflammatory disorders. However, since these approaches can lead to general immune suppression, strategies to inhibit the inflammasome by targeting NLRs and inflammatory caspases, may yield additional therapeutics.

Ferroptosis inhibitors show potential for the treatment of neurodegenerative diseases as an increase in iron is often associated with the induction of ferroptotic cell death. Conditions, such as stroke, ischemia-reperfusion damage, fibrosis, and inflammatory disease, all display cell death that has been successfully suppressed by ferroptosis inhibitors. Conversely, a ferroptosis inducer, like erastin, may prove to be useful in combating cancer. Tumor cells have already been shown to be susceptible to ferroptosis, so erastin, and compounds like it, may enhance cell death caused by chemotherapeutic agents. The role of ferroptosis in tumor progression and the potential for targeted treatments is a fascinating area of ongoing research.

Importantly, components of many cell death pathways can serve as key biomarkers as well as potential therapeutic targets. Together, protein targets in these cell death pathways offer tremendous promise for translational research and the treatment of multiple diseases.

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