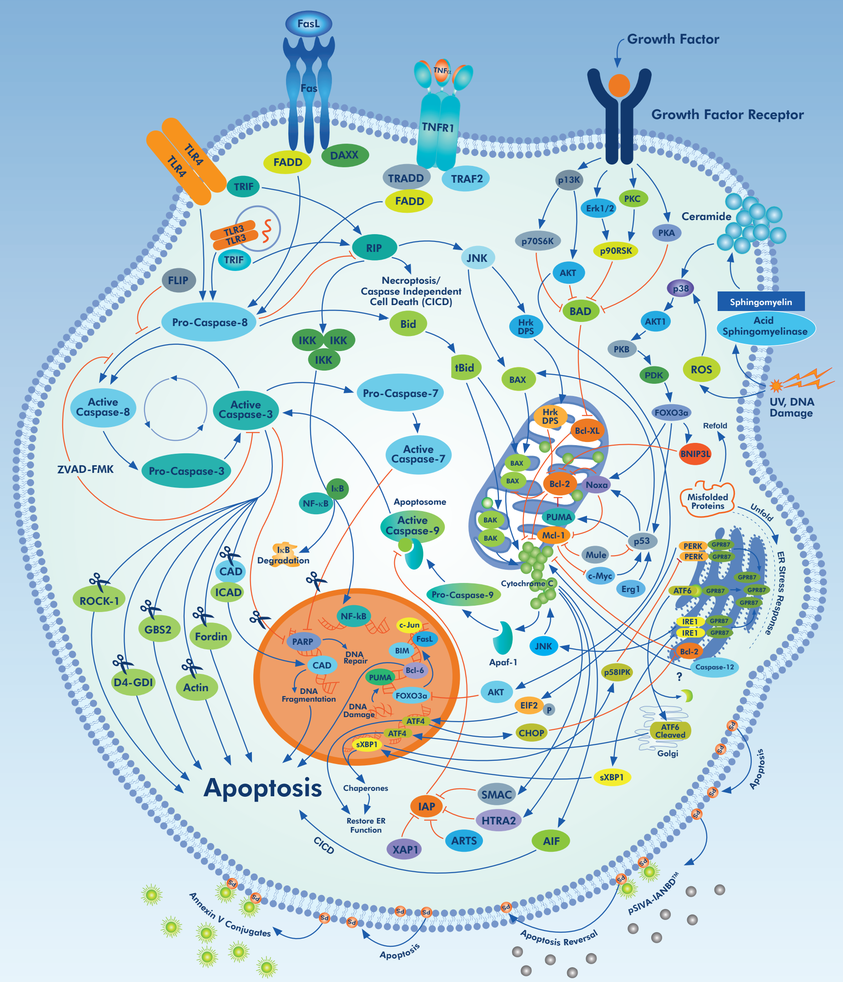
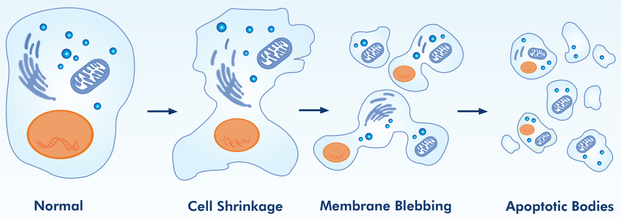
NOVUS BIOLOGICALS
Today's Research. Tomorrow's Discovery.

PATHWAY PROFILING ANTIBODIES & REAGENTS

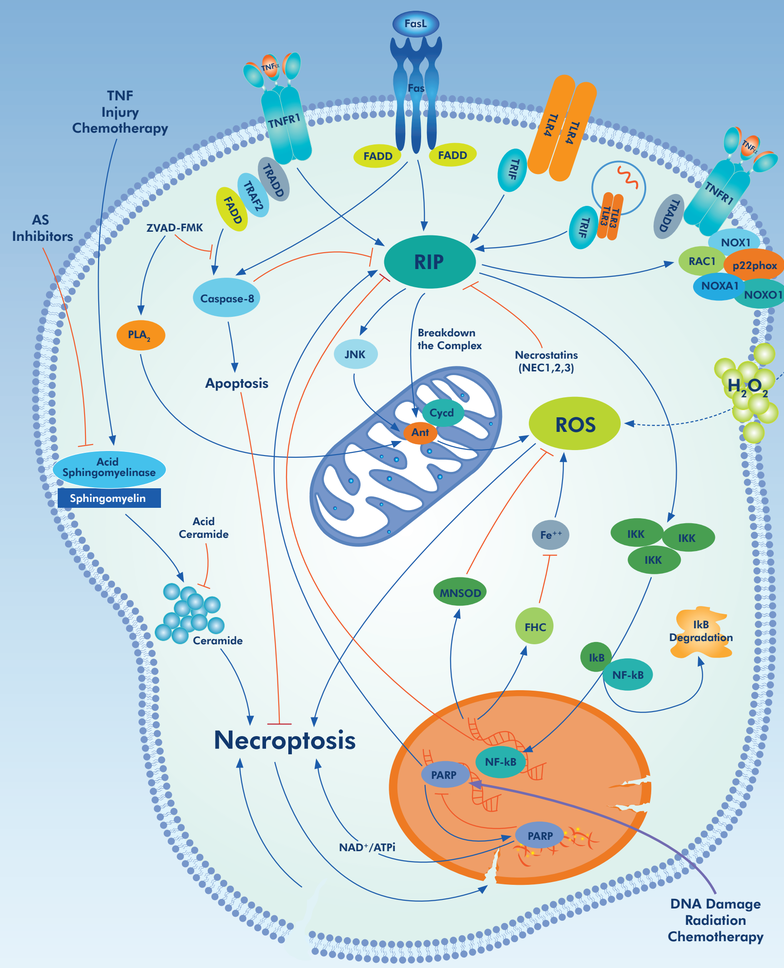
Apoptosis, Necroptosis & Autophagy

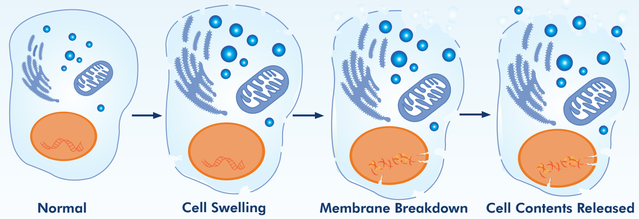
APOPTOSIS



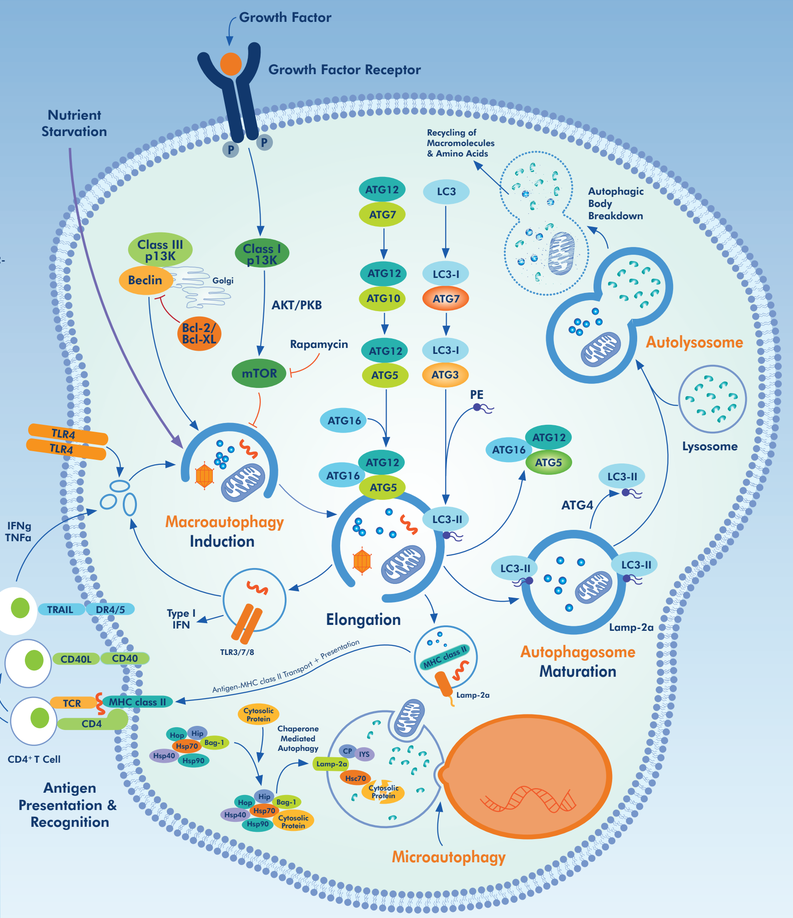


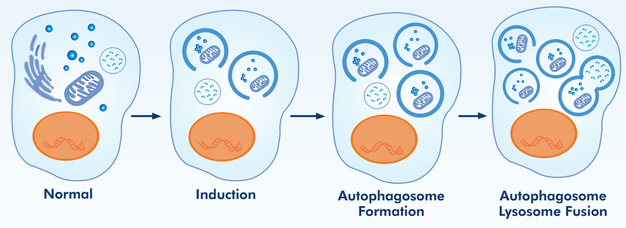
NECROPTOSIS





AUTOPHAGY





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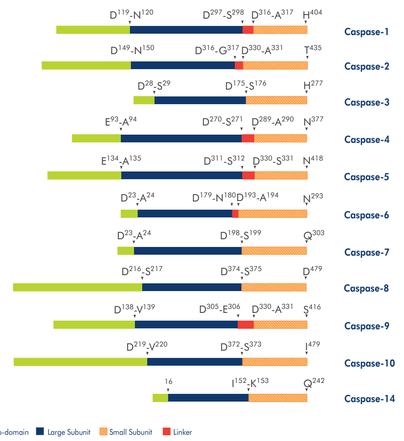
Cell Death Assays: According to the Recommendations of the Nomenclature Committee on Cell Death

The cell death field exploded during the 1990s and by the early 2000's assays to measure cell death were commonplace. New cell death types were routinely being elucidated and certain terms like 'apoptosis' became popularized. However descriptors like 'percent apoptosis' were vague, nomenclature lacked uniformity, and the cell death literature was becoming unwieldy. Hence in 2005, the editors of *Cell Death and Differentiation* formed the Nomenclature Committee on Cell Death (NCCD) which addressed the need to streamline cell death reporting. The NCCD guidelines are based on a series of measurable biochemical, functional and molecular features to characterize cell death subroutines. According to the NCCD, it is necessary to use at least two detection methods to precisely define an observed cell death.

Novus Biologicals is proud to facilitate the recommendations of the NCCD by offering a broad, comprehensive portfolio encompassing a myriad of methods for studying cell death-related phenomenon.

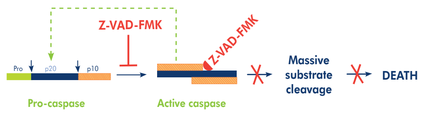
Galluzzi et al. Cell Death Differ. 12:1463-1467 (2005). Kroemer et al. Cell Death Differ. 19:107-120 (2012).

Caspase Structures



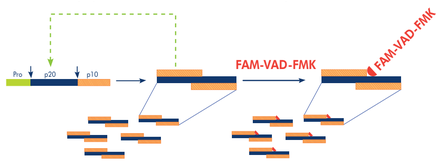
Caspases are synthesized as inactive pro-enzyme precursors containing an N-terminal prodomain of variable length followed large (p20) and small (p10) subunits. Caspases are activated through proteolytic cleavage (arrow symbols) at specific asparagine residues located within the pro-domain, and the p10 and p20 subunits. Activation results in the generation of mature active caspases consisting of heterotetramers p202-p102.

Apoptosis Inhibition with Caspase Inhibitors



Caspase inhibitors like pan caspase inhibitor Z-VAD-FMK (NBP2-29392) are used in in vitro and in vivo assays to inhibit apoptosis. These cell permeable peptides function by binding irreversibly to the catalytic or active site of caspases, thereby preventing the processing of pro-caspases to their fully active forms.

Apoptosis Detection with Labeled Caspase Inhibitors



Fluorescently labeled caspase inhibitors like FAM-VAD-FMK (NBP2-29383) are used in fluorescence microscopy and flow cytometry assays to detect apoptosis. Just like their unlabeled counterparts, they bind to the active site of caspases.

Cell Death Terms

- Accidental cell death
- Anoikis
- Aponecrosis
- Apoptosis
- Autolysis
- Autophagic cell death
- Autophagy
- Caspase-dependent intrinsic apoptosis
- Caspase-independent intrinsic apoptosis
- Cornification
- Entosis
- Excitotoxicity
- Extrinsic apoptosis by death receptors
- Extrinsic apoptosis by dependence receptors
- Mitotic catastrophe
- Necrosis
- Netosis
- Necroapoptosis
- Necroptosis
- Parthanatos
- Programmed cell death
- Pyroptosis
- Oncosis
- Wallerian degeneration

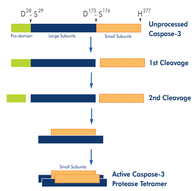
Antibody Detection of Pro and Active/Cleaved Caspases

Caspase antibodies are classical tools for detecting unprocessed (pro) and active (cleaved) forms of caspases by western blot. Western blot banding patterns will depend on the epitope recognized by a given antibody and the caspase forms present.

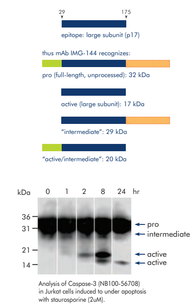
For example, the epitope of the Caspase-3 mAb (NB100-56708), clone 31A1067 has been mapped to the large subunit of Caspase-3 (between amino acids 29-176). Therefore, NB100-56708 recognizes pro-Caspase-3 (~32 kDa) and cleavage fragments containing the 17 kDa large subunit.

It is important to note that caspase cleavage fragments may be transient due to a short half-life and therefore loss of the caspase-3 pro-form without the appearance of cleavage fragments can also be an indication of caspase activation.

Caspase-3 Activation

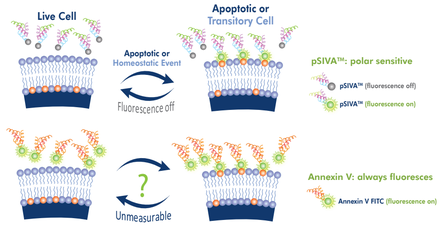


Caspase-3 Antibody



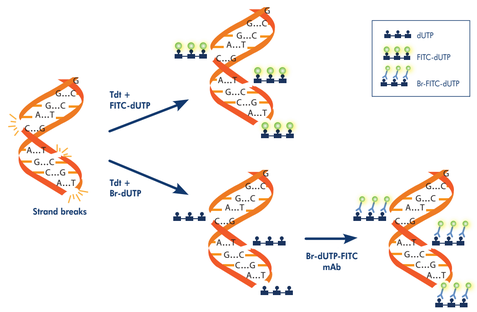
Analysis of Caspase-3 (NB100-56708) in Jurkat cells induced to under apoptosis with staurosporine (2uM).

pSIVA-IANBD™ versus Annexin V-FITC



pSIVA-IANBD™ [Polarity Sensitive Indicator of Viability (NBP2-29382/NBP2-29611)] and Annexin V-FITC (NBP2-29373) apoptosis technology. Both pSIVA-IANBD™ and Annexin V- fluorescent conjugates bind to exposed bind to exposed phosphatidylserine (PS) residues on cell surfaces, a hallmark of apoptosis. A key advantage of pSIVA-IANBD™ is that it fluoresces only when bound to cells, rendering it uniquely suitable for live microscopy imaging of PS exposure and monitoring apoptosis real time.

DNA Fragmentation TUNEL Apoptosis Assays

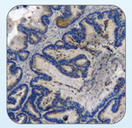


APO-BRDU™ (NBP2-31161, NBP2-31164) and APO-DIRECT™ (NBP2-31159) TUNEL Assays detect DNA fragmentation associated with apoptosis. TdT catalyzes the addition of Br-dUTP or FITC-dUTP to DNA strand breaks. FITC-dUTP is detected directly and Br-dUTP is detected with a BrdU-FITC mAb.

Apoptosis

Apoptosis is a naturally occurring, programmed cell death process involving a series of biochemical events leading to characteristic morphological changes and cellular demise. Apoptosis dysregulation has been implicated in a myriad of diseases.

BID
NB100-56106

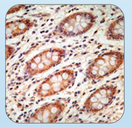


IHC-P: ovarian cancer

Bif-1
NBP2-24733

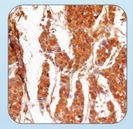
WB: Jurkat

Caspase-1
NB100-56565



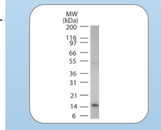
IHC-P: rectal cancer

Caspase 3
NB100-56708



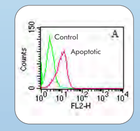
IHC-P: breast cancer

Cytochrome c
NB100-56503



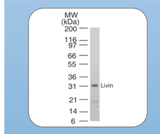
WB: HeLa

D4-GDI (Cleavage Specific)
NB100-56725



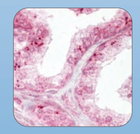
FLOW: HL60

Livin
NB100-56548



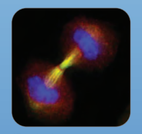
WB: Molt4

SMAC
NB100-56311



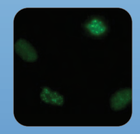
IHC-P: prostate

Survivin
NB500-201



IF: telophase

Survivin (pThr34)
NB500-236

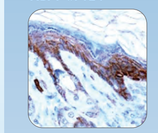


IF: HeLa

Autophagy

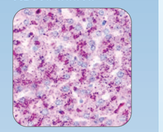
Autophagy is a basic catabolic mechanism involving lysosomal degradation and recycling of surplus or dysfunctional cellular components. Autophagy may promote cell survival or cell death depending on its execution context.

AMBRA1
NBP1-07124



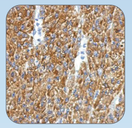
IHC-P: epidermis

ATG5
NB110-53818



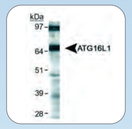
IHC-P: liver

ATG12
NBP2-15501



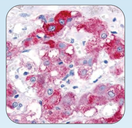
IHC-P: U87 xenograft

ATG16L1
NB110-60928



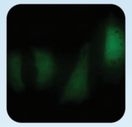
WB: HeLa

Beclin1
NB500-249



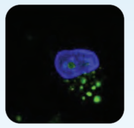
IHC-P: adrenal gland

LC3
NB100-2220



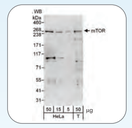
IF: Hek293

LC3b
NB600-1384



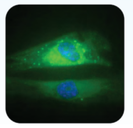
IF: U373-MG

mTOR
NB100-240



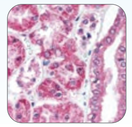
WB: HeLa, 293T

SQSTM1
NBP1-48320



IF: HeLa

ULK1
NBP2-24738

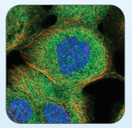


IHC (P): kidney

Necroptosis

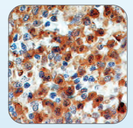
Necroptosis is the execution of necrosis, a form of fatal cell injury, through regulated cell signaling pathways. Necroptosis plays a role in various pathologies, particularly those involving inflammatory processes.

Caspase 4
NBP1-87681



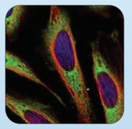
IF: A431

Caspase 8
NB100-56527



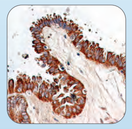
IHC-P: spleen

FADD
NBP2-16406



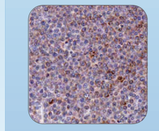
IF: HeLa

NFkB p65
NBP2-27416



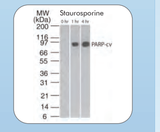
IHC-P: ovarian cancer

Noxa
NB600-1159



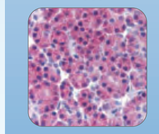
IHC-P: leukemia

PARP
NB100-56599



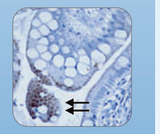
WB: Jurkat

RIPK3
NBP2-24588



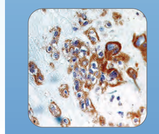
IHC-P: pancreas

TLR3
NBP2-24875



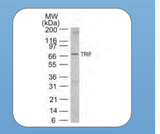
IHC-P: gut lumen

TNFAIP3
NBP1-77533



IHC-P: placents

TRIF
NB120-13810

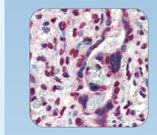


WB: Raw 264.7

UPR (Unfolded Protein Response)

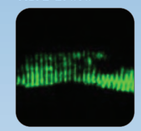
The UPR is a stress response activated in response to an accumulation of unfolded or misfolded proteins in the endoplasmic reticulum. Active UPR initiates signaling pathways to either correct the problem or promote apoptosis.

ATF6
NBP1-40256



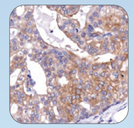
IHC-P: placenta

BAG3
NBP2-27398



IHC-F: muscle

Calnexin
NB100-1965



IHC-P: prostate

CHOP
NB600-1335

WB: HeLa

GRP78
NBP1-06274

IF: HeLa

HRD1
NB100-2526

IF: Hek293

IRE1 (pSER724)
NB100-2323

WB: Min6

XBP1
NBP1-77681

IHC-P: bladder

Caspase Inhibitors

Broad Spectrum: Boc-D(OMe)-FMK	NBP2-29395
Broad Spectrum: Z-VAD-(OMe)-FMK	NBP2-29392
Broad Spectrum: Q-VD-OPH	NBP2-29391
Caspase 3/7: Z-DEVD-FMK	NBP2-29396
Caspase 8: Z-IETD-FMK	NBP2-29397
Caspase 9: Z-LEHD-FMK	NBP2-29398
Negative Control: Z-FA-FMK	NBP2-29384

Cell Death Analysis Kits

Annexin V-FITC Kit [FITC]	NBP2-29373
APO-BRDU (TUNEL) Kit	NBP2-31161
APO-BRDU-IHC (TUNEL) Kit	NBP2-31164
APO-DIRECT (TUNEL) Kit	NBP2-31159
Caspase Kit [FAM-VAD-FMK]	NBP2-29383
Caspase Kit [SR-VAD-FMK]	NBP2-29399
Mitochondrial Membrane Potential Kit	NBP2-31093
NF-kB p65 ELISA Kit	NBP2-31042
pSIVA™ Flow Kit [IANBD]	NBP2-29611
pSIVA™ Microscopy Kit [IANBD]	NBP2-29382

Viability Dyes

7-AAD	NBP2-29446
Calcein Blue, AM	NBP2-31158
Calcein, AM	NBP2-31157
DAPI	NBP2-31156
Propidium Iodide	NBP2-31155

Extraction Kits

Endoplasmic Reticulum Enrichment Kit	NBP2-29482
Mitochondrial Extraction Kit	NBP2-29448
Nuclear Extraction Kit	NBP2-29447

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