



A GUIDE TO

CELL DEATH MECHANISMS & DETECTION TOOLS

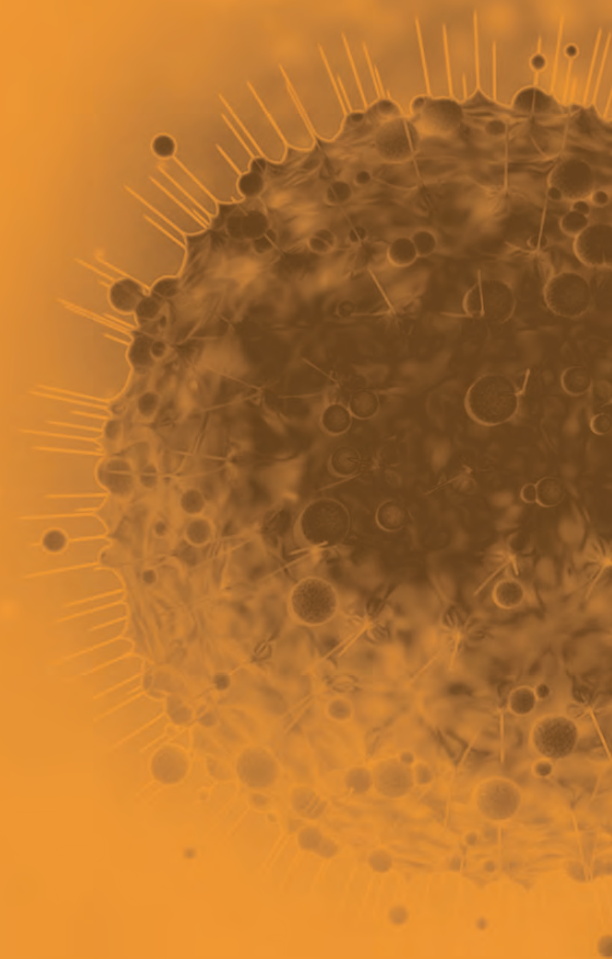
Cell death occurs for both physiological and pathological reasons. Varying modes of death are either programmed to safely remove healthy cells that are no longer useful or to stimulate an inflammatory response to clear cells that are useful but no longer healthy.

To distinguish among the multiple ways a cell can self-destruct, distinct morphological changes, triggers, and biochemical hallmarks are monitored. However, crosstalk between certain modes of death can also occur, making it important to examine more than one readout when identifying the types of cell death in your experiments.

Use this guide to learn about the tools available to investigate multiple molecular pathways and cell death markers to help you determine the cell death mechanism(s) occurring under your experimental circumstances.

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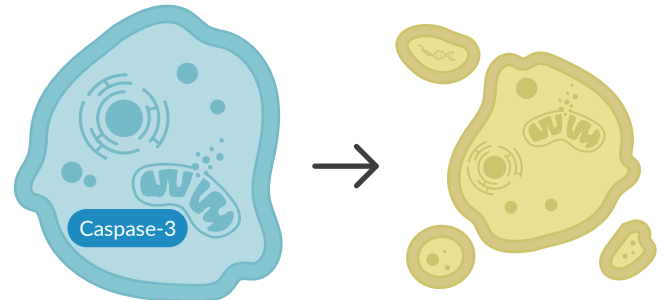
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APOPTOSIS

A tightly controlled, ATP-dependent form of programmed cell death that does not induce an inflammatory response as the cell membrane remains intact. Characterized by distinct morphological changes along with the activation of specific caspases and mitochondrial control pathways that serve as markers of the process.

- Activation of apoptosis signaling cascades
- Pannexin channel “find me” signal release
- Phosphatidylserine (PS) exposure
- Mitochondria outer membrane permeability
- Release of cytochrome c from mitochondria
- Activation of initiator and effector caspases
- Cleavage of specific caspase substrates
- Chromatin condensation and DNA fragmentation
- Membrane blebbing
- Formation of apoptotic bodies

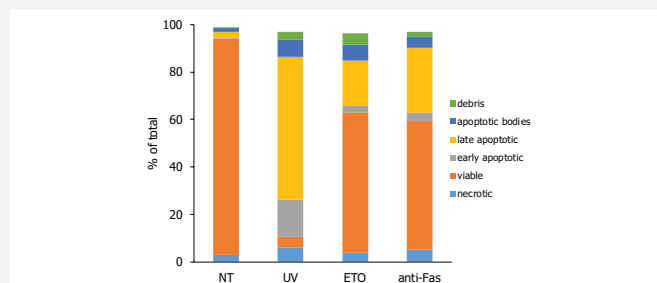


Simultaneous Detection of Multiple Changes in Apoptosis Pathways

Because cell death can occur by several different paths that may share similar characteristics, multiple apoptosis markers should be examined to confirm this mechanism of cell death in your experimental system.

Early Apoptosis Detection Assay Kit

Item No. 601360



Cell populations induced to undergo apoptosis by various stimuli: Untreated (NT); Ultraviolet light (UV); Etoposide (ETO); Anti-Fas CH-11

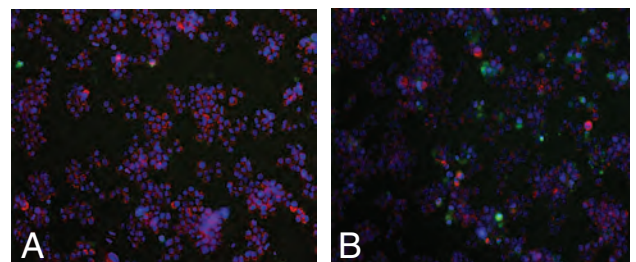
Contains

DAPI - DNA stain measures membrane permeability
 Annexin V - PS exposure and loss of membrane asymmetry
 TMRE - Mitochondrial membrane potential
 TO-PRO®-3 - Identifies active pannexin channels

See how this kit can be used for high-throughput flow cytometric screening at www.caymanchem.com/HTPapoptosisFC

Multi-Parameter Apoptosis Assay Kit

Item No. 601280



A) Untreated. B) RAW 264.7 cells stimulated to undergo apoptosis by UV concurrently decrease TMRE staining and increase Annexin V FITC staining.

Contains

Hoechst 33342 - Demonstrates nuclear morphology
 Annexin V - PS exposure and loss of membrane asymmetry
 TMRE - Mitochondrial membrane potential
 RedDot™2 - DNA stain measures membrane permeability

Learn more about how to choose the right apoptosis assay for your research at www.caymanchem.com/apoptosis

Pro- and Anti-Apoptotic Signaling Pathways

The pro- and anti-apoptotic Bcl-2 family of proteins regulates commitment to cell death through controlling mitochondrial integrity. Cleavage or oligomerization as well as the abundance of these proteins can mark apoptosis.

Bax Modulators

| Item No. | Product Name |
|----------|--------------|
| 14128 | Fludarabine |
| 15559 | Mdivi 1 |
| 19230 | Nimbolide |

[View additional Bax modulators online at www.caymanchem.com](http://www.caymanchem.com)

Bcl-2 Family Inhibitors

| Item No. | Product Name |
|----------|--------------------------|
| 16233 | ABT-199 |
| 11314 | Chelerythrine (chloride) |
| 21478 | WEHI-539 |

[View additional Bcl-2 inhibitors online at www.caymanchem.com](http://www.caymanchem.com)

Cell Membrane Alterations

PS migrates to the outer plasma membrane in apoptosis, causing a loss of membrane asymmetry. Annexin V binds to exposed PS and can be paired with a membrane-impermeable dye to distinguish between intact, apoptotic, and necrotic cells.

Annexin V Assays

| Item No. | Product Name | Includes | Ex/Em Filters |
|----------|--------------------------|-----------------------------------|-------------------------------------|
| 601410 | Annexin V APC Assay Kit | APC-conjugated annexin V and DAPI | 633/700 nm (APC); 350/450 nm (DAPI) |
| 600300 | Annexin V FITC Assay Kit | FITC-conjugated annexin V and PI | 488/525 nm (FITC); 655-730 nm (PI) |
| 601420 | Annexin V PE Assay Kit | PE-conjugated annexin V and DAPI | 488/585 nm (PE); 350/450 nm (DAPI) |

Mitochondrial Changes

Mitochondrial outer membrane permeabilization causes a decrease in transmembrane potential ($\Delta\psi_m$) and the release of cytochrome c. $\Delta\psi_m$ is assessed using positively charged dyes, such as JC-1 and TMRE, that accumulate inside active mitochondria.

Mitochondrial Membrane Potential Assays

| Item No. | Product Name |
|----------|---|
| 10009172 | JC-1 Mitochondrial Membrane Potential Assay Kit |
| 701310 | TMRE Mitochondrial Membrane Potential Assay Kit |

Caspase Activation

Release of cytochrome c from the mitochondria promotes caspase-9 activation *via* Apaf-1, which then activates caspases-3 and -7. Caspase activation can be confirmed by detecting cleaved PARP1 or fluorogenic substrates.

Caspase Activity Assay & Related Antibody

| Item No. | Product Name |
|----------|------------------------------------|
| 10009135 | Caspase-3/7 Fluorescence Assay Kit |
| 13557 | PARP (Cleaved) Monoclonal Antibody |

Chromatin Condensation & DNA Fragmentation

The condensation of chromatin is accompanied by the hydrolysis of nuclear DNA into a ladder of fragments that can be detected by gel electrophoresis. The 3' ends of DNA fragments can also be labeled with deoxyuridine. Cell-permeable Hoechst 33342 is often used to identify nuclear condensation by microscopic analysis.

DNA Fragmentation Detection

| Item No. | Product Name |
|----------|-------------------------|
| 15580 | 5-Bromo-2'-deoxyuridine |
| 660990 | DNA Laddering Kit |

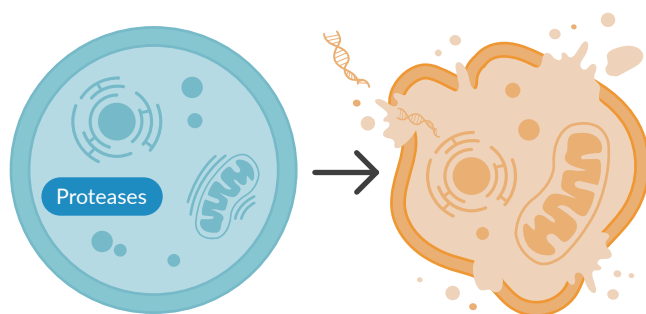
Nuclear Condensation Detection

| Item No. | Product Name |
|----------|-------------------------------|
| 15547 | Hoechst 33342 (hydrochloride) |

NECROSIS

Classically thought of as unprogrammed cell death and characterized by cell swelling and an eventual break of the cell membrane. An inflammatory response occurs due to a leak of cellular contents, unlike apoptosis where cellular contents are neatly packaged.

- No externalization of PS
- Activation of calcium-dependent proteases
- Mitochondrial swelling
- No chromatin condensation
- DNA is randomly digested
- Loss of membrane integrity
- Total cell lysis



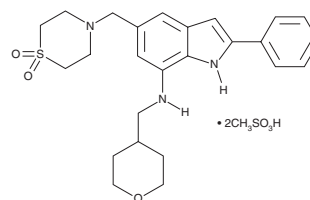
Cell-Impermeable DNA Stains

| Item No. | Product Name |
|----------|---------------------------|
| 11397 | 7-Aminoactinomycin D |
| 14285 | DAPI (hydrochloride) |
| 25170 | Nuclear Blue™ DCS1 |
| 25171 | Nuclear Green™ DCS1 |
| 25173 | Nuclear Orange™ DCS1 |
| 25175 | Nuclear Red™ DCS1 |
| 14289 | Propidium iodide |
| 10008351 | Propidium iodide Solution |

Cell-Permeable Necrosis Inhibitor

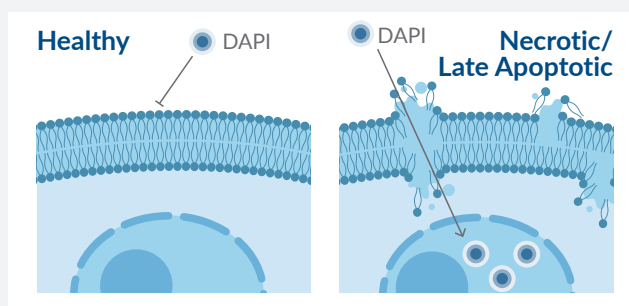
Necrox-5 (methanesulfonate) - Item No. 17278

Predominantly localizes in mitochondria, selectively blocking oxidative stress-induced necrotic cell death.



Dye Exclusion Test

The damaged membranes of cells in necrosis or late apoptosis enable the uptake of membrane-impermeable dyes like 7-AAD, propidium iodide, DAPI, or RedDot™2. Viable cells remain unstained because intact membranes exclude these dyes.



Several regulated necrotic death pathways have been identified: necroptosis, pyroptosis, ferroptosis, and NETosis. Flip through the following pages to learn more about these pathways.

Cell Death Screening Library - Item No. 35093

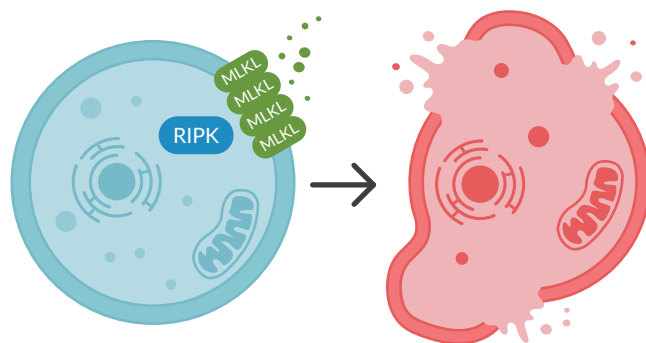
- Includes ~152 inducers and inhibitors of multiple cell death pathways



NECROPTOSIS

A host defense mechanism against pathogens that can be induced through death receptor ligand binding. Mediated by activation of receptor-interacting protein kinase 3 (RIPK3) and its substrate, mixed lineage kinase domain-like (MLKL). Controlling the phosphorylation states of RIPK1, RIPK3, and MLKL is a key step to controlling necroptosis. Caspase-8 can inhibit the process by cleaving RIPK1 and RIPK3 and potentially pivot the cell towards a caspase-driven, apoptotic process instead.

- Occurs independent of caspase activation
- RIPK3 is activated in complex with RIPK1
- RIPK3 phosphorylates MLKL
- MLKL generates a membrane pore complex
- Damage-associated molecular patterns (DAMPs) released
- PS exposure
- Cell swelling
- Plasma membrane rupture



RIPK Inhibitors

| Item No. | Product Name |
|----------|-------------------|
| 20308 | GSK481 |
| 23300 | GSK872 |
| 11658 | Necrostatin-1 |
| 20924 | (±)-Necrostatin-2 |
| 10527 | Necrostatin-5 |

View additional RIPK inhibitors online at www.caymanchem.com

MLKL Inhibitors

| Item No. | Product Name |
|----------|------------------|
| 27431 | Ligustroflavone |
| 20844 | Necrosulfonamide |

Caspase-8 Activity Detection

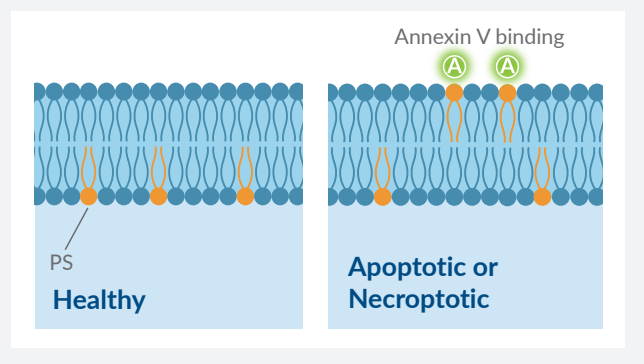
| Item No. | Product Name | Ex/Em Filters |
|----------|---|-----------------------|
| 17480 | Ac-IETD-AFC | 400/505 nm |
| 27139 | Ac-IEPD-pNA (trifluoroacetate salt) | 405 nm (colorimetric) |
| 14991 | Ac-LETD-AFC | 400/505 nm |
| 27101 | Z-IETD-AFC | 400/505 nm |
| 28212 | (Z-IETD) ₂ -Rh 110 (trifluoroacetate salt) | 496/520 nm |

Caspase-8 Inhibitors

| Item No. | Product Name |
|----------|---|
| 27438 | Ac-AAVALLPAVLALLAP-IETD-CHO (trifluoroacetate salt) |
| 27100 | Ac-IETD-CHO (trifluoroacetate salt) |

PS Flip

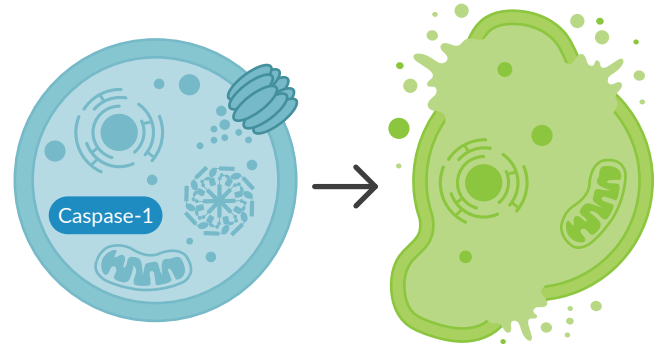
PS lipids can become exposed on the outer membrane of the cell during necroptosis just as they do during apoptosis. Turn back to page 3 to find annexin V assays that will detect exposed PS.



PYROPTOSIS

Activated in innate immune cells in the presence of pathogen-associated molecular patterns (PAMPs) or cell-derived DAMPs. NF- κ B activation leads to inflammasome formation. Inflammatory caspases (1, 4, 5, 11) enable gasdermin D cleavage, whose oligomers form a lytic pore in the cell membrane. Preventing inflammasome activation by inhibiting NLRP3 can control pyroptosis and limit the release of inflammatory cytokines.

- NF- κ B-induced expression of inflammasome proteins
- NLRP3, ACS/TMS1, and procaspase-1 form inflammasome
- Caspase-1 (or non-canonical caspase-4, -5, -11) activation
- Gasdermin D cleavage and pore formation
- Release of pro-inflammatory IL-1 β and IL-18
- Fluid intake and cell swelling
- Rapid plasma membrane rupture



NLRP3 Inhibitors

| Item No. | Product Name |
|----------|--------------|
| 33353 | CY-09 |
| 24671 | Dapansutrile |
| 28476 | INF39 |
| 17510 | MCC950 |
| 30895 | NLRP3i |

Caspase-1 Detection

Caspase-1 is proteolytically activated before regulating pro-inflammatory cytokines. Detecting cleaved caspase-1 can be used to monitor pyroptosis.

Caspase-1 Monoclonal Antibody (Clone 14F468)
Item No. 13907

IL-1 β Detection

Interleukin-1 β (human) TR-FRET Biomarker Assay Kit

Item No. 500230

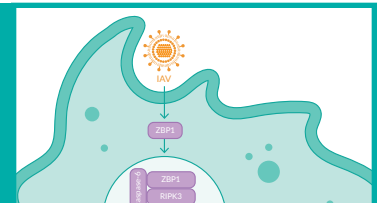
- A homogenous TR-FRET sandwich immunoassay method
- Measure in cell culture supernatants
- Assay Range: 32-10,000 pg/ml; LLOQ of 143 pg/ml
- Incubation: 2 hours
- Read: at 665 nm or the 665/615 nm ratio

Caspase-1 Inhibitors & Screening Assay

| Item No. | Product Name |
|----------|---|
| 10016 | Ac-YVAD-CHO |
| 10014 | Ac-YVAD-CMK |
| 27420 | YVAD-CHO (trifluoroacetate salt) |
| 701840 | Caspase-1 Inhibitor Screening Assay Kit |

View additional caspase-1 inhibitors online at www.caymanchem.com

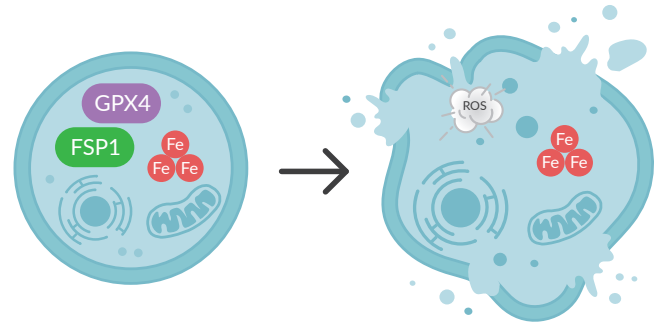
Cell death pathways do not always operate in isolation from one another. A newly described inflammatory programmed cell death pathway, PANoptosis, involves the collective activation of apoptosis, necroptosis, and pyroptosis. Find out more about this process at www.caymanchem.com/PANoptosis.



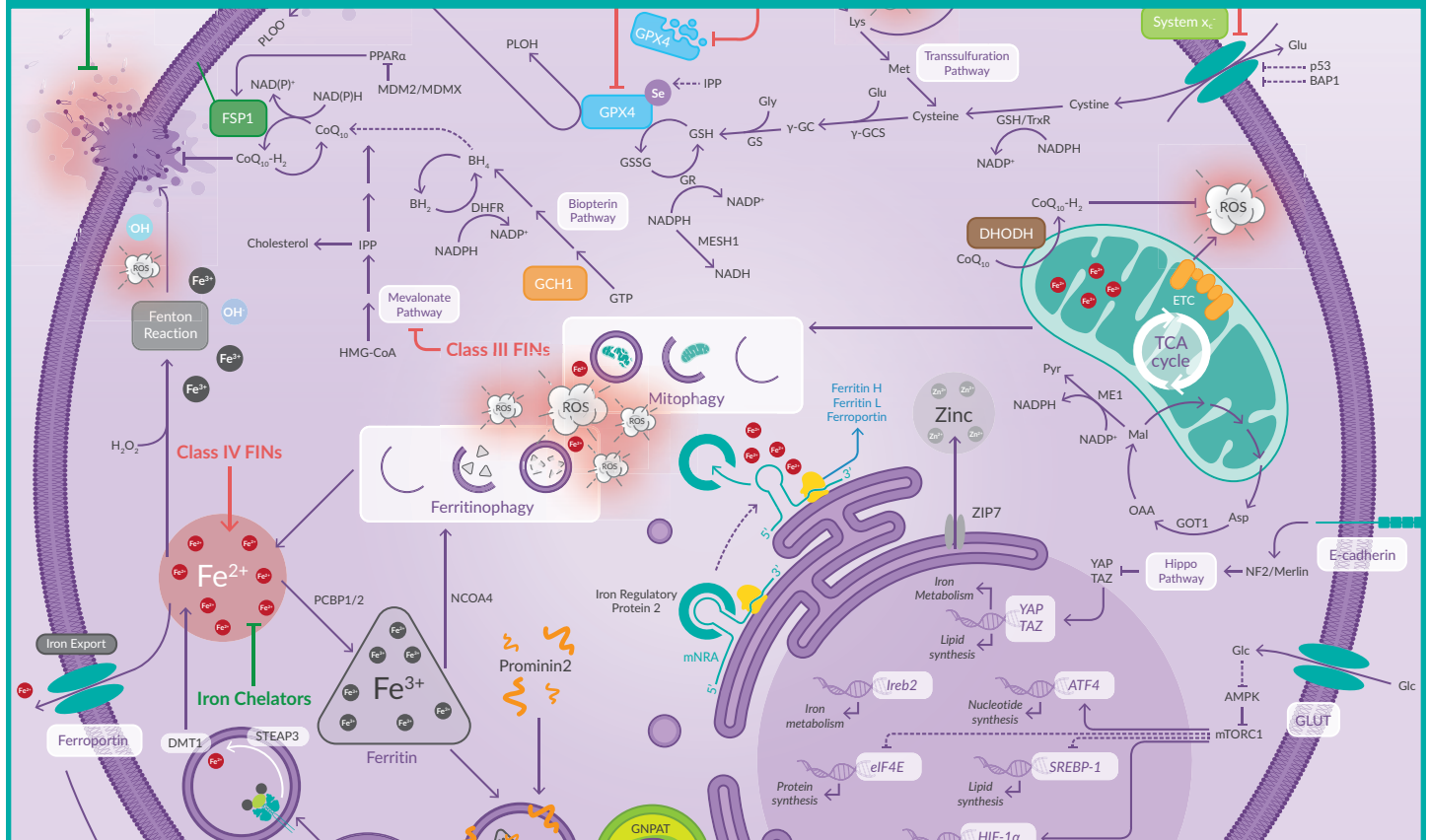
FERROPTOSIS

An iron-dependent cell death that results in an increase in lipid peroxides. Regulated by multiple cellular metabolic pathways, including redox homeostasis, iron handling, mitochondrial activity, and metabolism of amino acids, lipids, and sugars. Ferroptosis inducers (FINs) can affect glutathione peroxidase 4 (GPX4) or the ferroptosis suppressor protein 1 (FSP1), resulting in a decrease in antioxidant capacity and accumulation of lipid reactive oxygen species (ROS) in cells.

- Redox-active metal ions (labile iron pool) accumulate
- Endogenous antioxidants (GSH) depleted
- Phospholipid peroxidation increased
- Intracellular NAD(P)H depleted
- Antioxidant defense via Nrf2 signaling activated
- Ferritin and GPX4 degraded
- FSP1 inactivated
- Cell swelling with reduced mitochondrial volume
- Plasma membrane rupture



Learn more about the ferroptotic pathway and request a free wall poster. www.caymanchem.com/ferroptosis



Ferroptosis Induction

Small molecules that interfere with GSH production, suppress GPX4 or FSP1 activity, or oxidize ferrous iron, leading to degradation of the endoperoxide to induce lipid peroxidation, have been used to induce ferroptosis and promote the accumulation of ROS. Novel inducers can be discovered using GPX4 and FSP1 inhibitor screening assays.

Classic FINs

| Item No. | Product Name |
|----------|--------------------------------|
| 14484 | L-Buthionine-(S,R)-Sulfoximine |
| 17754 | Erastin |
| 25180 | FIN56 |
| 25096 | FINO ₂ |
| 29483 | iFSP1 |
| 20455 | ML-162 |
| 19288 | (1S,3R)-RSL3 |

[View additional FINs online at www.caymanchem.com](http://www.caymanchem.com)

Iron Transport

| Item No. | Product Name |
|----------|--|
| 32030 | Transferrin (human, recombinant) |
| 32311 | Transferrin Receptor/CD71 Rabbit Monoclonal Antibody |

Lipid ROS Detection

Cellular lipoxygenases such as 15-LO or the cytochrome P450 oxidoreductase as well as non-enzymatic processes driven by the Fenton reaction oxidize phospholipids. Detecting lipid peroxidation either directly or *via* known end-product reactive aldehydes can be used to monitor ferroptosis.

Lipid ROS Assays & Probes

| Item No. | Product Name |
|----------|--|
| 501140 | DHN-MA EIA Kit |
| 705002 | Lipid Hydroperoxide (LPO) Assay Kit |
| 601290 | ROS Detection Cell-Based Assay Kit (DHE) |
| 27086 | C11 BODIPY 581/591 |
| 62237 | DPPP |

[View additional ROS detection tools online at www.caymanchem.com](http://www.caymanchem.com)

Ferroptosis Suppression

Radical-trapping antioxidants terminate radical chain reactions in phospholipids, while iron chelators remove excess iron, preventing the formation of highly reactive hydroxyl radicals.

Antioxidants

| Item No. | Product Name |
|----------|----------------|
| 17729 | Ferrostatin-1 |
| 17730 | Liproxstatin-1 |
| 26819 | Vatiquinone |

Inhibitor Screening Assays

| Item No. | Product Name |
|----------|--|
| 701900 | FSP1 Fluorescent Inhibitor Screening Assay Kit |
| 701880 | GPX4 Inhibitor Screening Assay Kit |

GSH Detection & GSH Redox Activity

| Item No. | Product Name |
|----------|--|
| 600360 | Glutathione Cell-Based Detection Kit (Blue Fluorescence) |
| 703202 | Glutathione Reductase Assay Kit |

[View additional GSH assays online at www.caymanchem.com](http://www.caymanchem.com)

Fluorescent Iron Indicators

| Item No. | Product Name |
|----------|-------------------------------|
| 25393 | Phen Green SK diacetate |
| 31095 | Rhodamine dithenoyl hydrazide |

OXIDIZED PHOSPHOLIPID LIPIDOMIC SERVICES

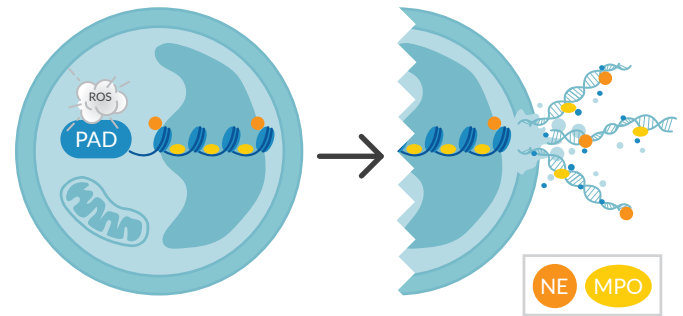
A targeted panel of phospholipids containing oxidized acyl chains (*e.g.*, 20:4-OH, 20:4-OOH) will help identify hydroperoxy and hydroxy phospholipids in your samples.

www.caymanchem.com/lipidomics

NETOSIS

A cell death specific to neutrophils and associated with host defense against pathogens or various inflammatory and autoimmune disorders. As the cell membrane ruptures, chromatin, histones, and antimicrobial cytoplasmic granule contents are extruded in neutrophil extracellular traps (NETs) in order to ensnare and kill extracellular pathogens. Chromatin decondenses due to protein arginine deiminase (PAD) hypercitrullination of histones. Neutrophil elastase (NE) and myeloperoxidase (MPO) induce cytoskeletal disassembly and histone degradation.

- PAD activation by ROS
- Citrullinated histone H3 (CitH3) production
- Chromatin decondensation
- NE and MPO induce histone degradation
- Cell membrane rupture
- Chromatin, CitH3, MPO, NE, and defensin released in NETs



Preferred Methods to Detect and Quantify NETs

Most NET components are also released in soluble form from neutrophil granules (e.g., MPO, NE, defensins) or during necrotic cell death (e.g., DNA, histones). NET-specific analytes such as CitH3 or induction of NETosis *ex vivo* best serve as accurate surrogates for measurement of NET formation.

Citrullinated Histone H3 (Clone 11D3) ELISA Kit - Item No. 501620

Quantify a NET-specific biomarker

- Detects CitH3, the one NET component that is produced almost exclusively during NET formation
- Validated in mouse bone marrow leukocytes and serum as well as human plasma and serum

NETosis Assay Kit - Item No. 601010

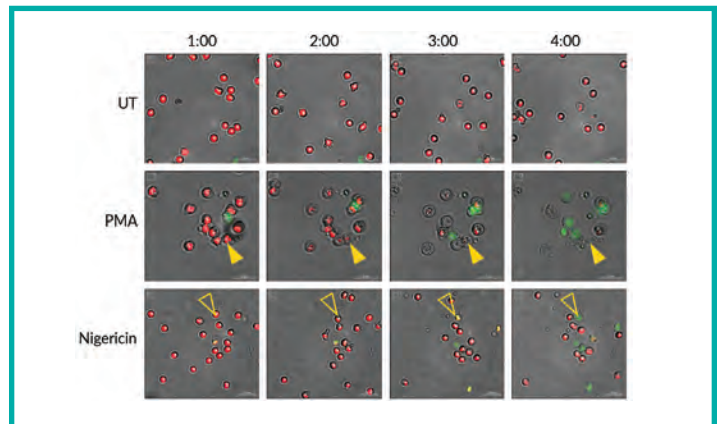
Study the process of NETosis *ex vivo*

- Measures elastase released from stimulated neutrophils
- Compatible with any source of NET-producing cells

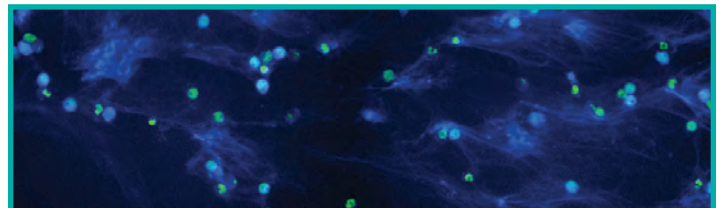
NETosis Imaging Assay Kit - Item No. 601750

Simple staining protocol for visualizing the process of NETosis kinetically *ex vivo* and *in vitro*

- Cell-permeable red DNA stain captures dynamic changes to nucleus
- Cell-impermeable green stain detects extruded DNA



Watch the time-lapse video for NETosis kinetics
www.caymanchem.com/imageNETs



Determine which NET assay is right for your experiment
www.caymanchem.com/quantifyNETs

PAD Detection & Inhibitor Screening

| Item No. | Product Name |
|----------|--|
| 501460 | PAD4 (human) ELISA Kit |
| 701320 | PAD4 Inhibitor Screening Assay Kit (AMC) |

View a complete list of research tools to study citrullination online at www.caymanchem.com

Activation/Isolation of Neutrophils

NETosis can be induced by bacteria, LPS, or calcium ionophores. Neutrophils can be enriched from human blood or mouse bone marrow or peritoneum by density centrifugation for downstream applications.

| Item No. | Product Name |
|----------|---|
| 11016 | A23187 |
| 10004974 | Ionomycin |
| 19660 | LPS from <i>Escherichia coli</i> O55:B5 |
| 10008014 | Phorbol 12-myristate 13-acetate |
| 601070 | Neutrophil (mouse) Isolation Kit |

NE & MPO Activity Screening

| Item No. | Product Name |
|----------|---|
| 600610 | Neutrophil Elastase Activity Assay Kit |
| 600620 | Neutrophil Myeloperoxidase Activity Assay Kit |

View a complete list of research tools to study neutrophil function online at www.caymanchem.com

NET FORMATION SCREENING & ANALYSIS SERVICES

Detection of NET formation *ex vivo* and screening for modulators using:

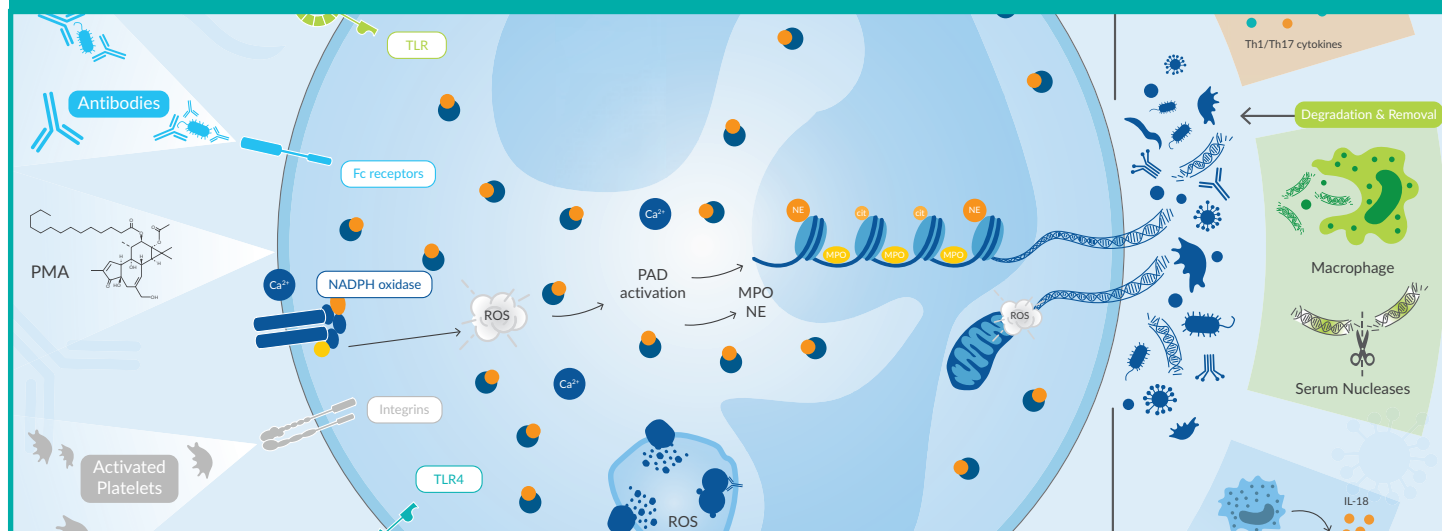
- High-content imaging
- Enzymatic detection
- Citrullination/carbamylation detection
- Experienced scientists skilled in neutrophil biology, isolation, and handling

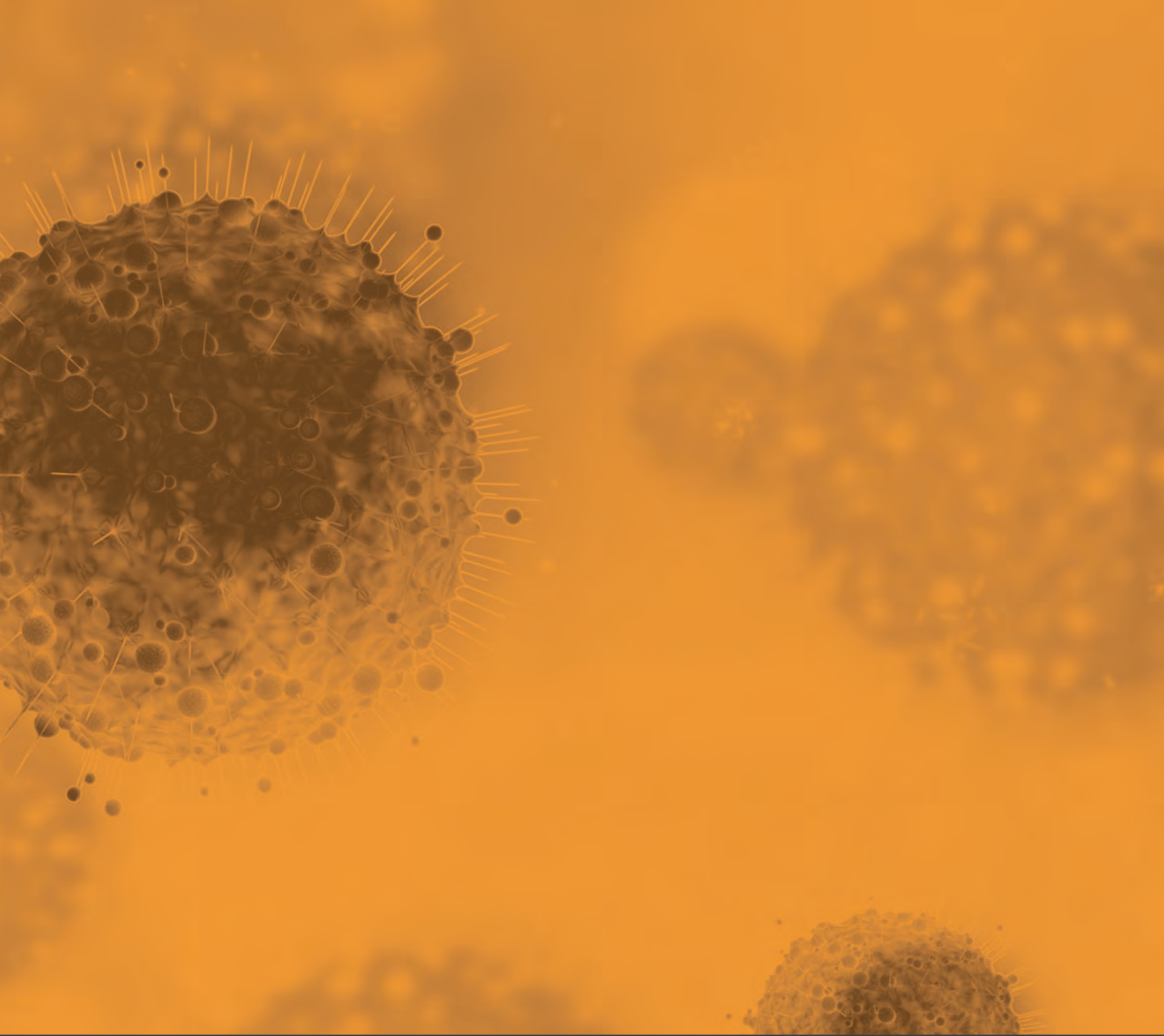
www.caymanchem.com/bioanalysis

NETosis Screening Set - Item No. 35019

- Contains ~40 compounds
- Includes a variety of published and potential NETosis stimulators and inhibitors
- Use with Cayman's NETosis Imaging Assay Kit (Item No. 601750) or NETosis Assay Kit (Item No. 601010) to evaluate NET production *in vitro*

Request a Neutrophil Biology wall poster to view the events of NETosis and biological aspects of the neutrophil that relate to the process.
www.caymanchem.com/neutrophilposter





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