EMD MILLIPORE

Life, death, and everything in between.

Comprehensive solutions for studying cell health.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Platforms, Technologies, and Services

As a tools provider and partner in research, EMD Millipore is committed to the advancement of life science research and therapeutic development. This guide includes a number of new products for target identification, pathway detection, and profiling. These products provide proven solutions for a range of applications and are backed by extensive technical support.

CALBIOCHEM® SMALL MOLECULES

Small-molecule compounds, including inhibitors, activators, and other pathway modulators, are critical tools for researchers studying cell signaling and other mechanisms that control cell fate, function and phenotype. EMD Millipore's Calbiochem® reagents have been cited in thousands of peer-reviewed publications. From libraries and pathway panels to individual reagents, the Calbiochem® line of products offers the widest and most cited selection of inhibitors and activators worldwide.

CELLS AND CELL CULTURE

EMD Millipore's innovative cell culture solutions help optimize cell growth and maintenance. We offer an extensive range of human and rodent stem cells, primary cells and media designed for most types of stem cells, including embryonic, mesenchymal, and neural stem cells. These optimized media include serum-free, feeder-free formulations, and are supported by our feeder cells, supplements, reagents, and cultureware. Our flexible sterile filtration devices offer fast flow and have many membrane options. Also available are membrane-based cultureware to mimic *in vivo* conditions and provide coculture options.

ANTIBODIES AND IMMUNOASSAYS

With the expertise of Upstate® and Chemicon®, EMD Millipore provides an extensive, focused, validated portfolio of antibodies and immunoassays, with breadth and depth in major research areas backed by excellent service and support. EMD Millipore also offers a variety of ELISAs in major research areas, including cell health.

CELL-BASED ASSAYS AND QUANTITATIVE CELL IMAGING

Our portfolio of live cell, whole-cell and cell-based activity assays and reporter systems advances direct and indirect detection of biological processes. These technologies facilitate protein target validation, identify cellular pathways and determine mechanism of action for lead optimization environments. EMD Millipore also offers assays for high-content, multiparametric cell imaging, enabling identification of cellular responses and events under user-defined conditions.

FLOW CYTOMETRY ASSAYS AND SYSTEMS

Flow cytometry is essential for in-depth cell analysis, with the capacity to simultaneously measure multiple parameters on individual cells. Our easyCyte™ flow cytometers provide direct, precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. Our validated FlowCellect™ assay kits and Milli-Mark™ conjugated antibodies, along with application-specific analysis software modules, provide a complete solution for flow cytometry.

MILLIPLEX® MAP MULTIPLEX ASSAYS

MILLIPLEX® MAP assays offer the broadest selection of multiplex kits and reagents in a wide variety of research areas, measuring multiple biomarkers using a small sample size. MILLIPLEX® MAP enables the simultaneous detection of multiple soluble or intracellular biomarkers. Using the Luminex® xMAP® bead-based technology, these flexible and customizable assays are exhaustively tested and qualified for sensitivity, specificity, reproducibility and wide dynamic range.

MOLECULAR BIOLOGY TOOLS

For every step of the molecular biology and protein workflow, from cloning DNA targets to purifying native recombinant proteins, EMD Millipore provides reagents, kits, cells and tools that are specifically designed to meet your scientific and technical goals, including the Novagen® line of products for DNA amplification, purification, and propagation and reagents for efficient transfection.

Introduction

Cells maintain a healthy state by delicately balancing multiple processes and signaling pathways. Only by studying all of these facets of cell health can we accurately characterize the state of a cell under specified conditions. Diverse inputs, such as disease states, nutrient availability, cell-cell contacts, extracellular matrix, tissue microenvironment, oxygen content, developmental cues, or various forms of cellular stress can shift the balance of cell health pathways. Therefore, a multipronged approach using complementary analysis platforms and technologies is necessary for a complete picture of cell health.

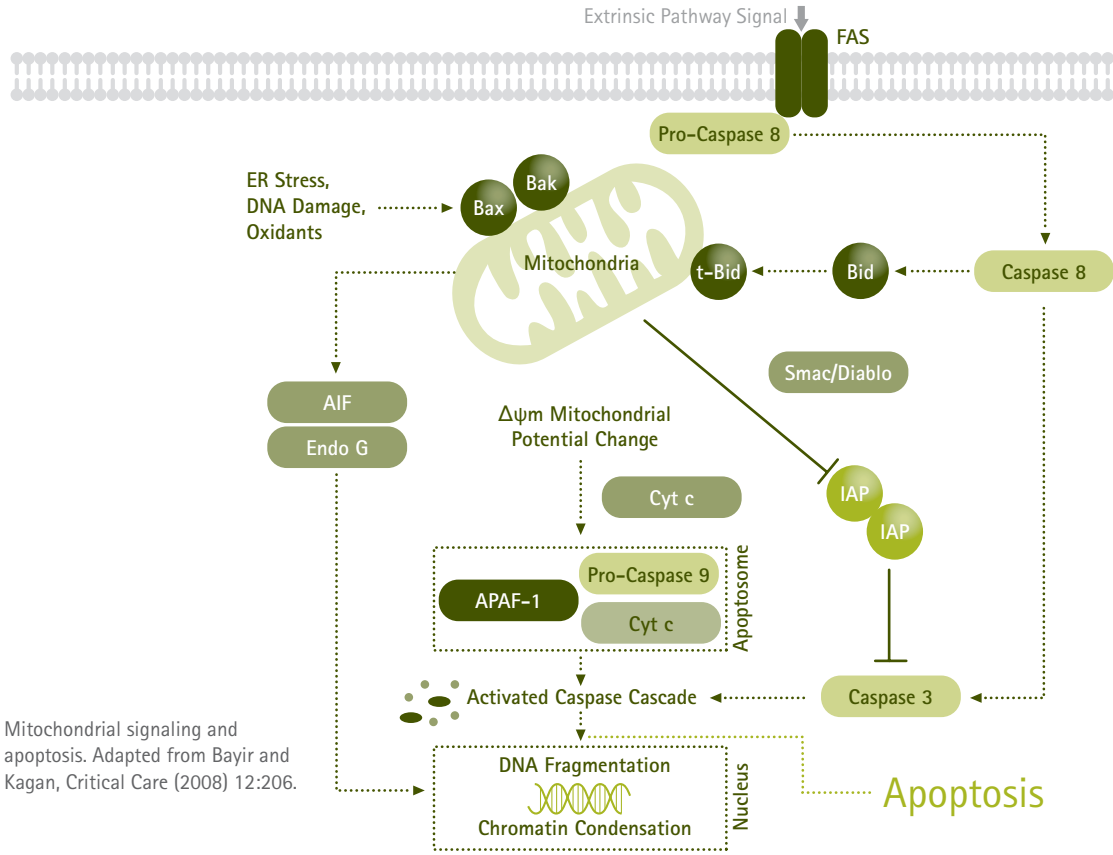
In addition to using cell health assessment for elucidating disease mechanisms and therapeutic discovery, monitoring key indicators of cell health and performance (such as the apoptotic fraction, viability, cell cycle, cell counts, and transfection efficiency) helps establish uniform standards of cellular performance across long-term research studies. Knowing the performance profile of your cells prior to running your bioassay can mean the difference between valid assay results and wasted reagents, lost time and discarded data.

Count on EMD Millipore's trusted, well-published antibodies, small molecules, assays and kits, along with our innovative platforms and technologies for cellular and protein analysis to accelerate your studies of cell health.

Table of Contents

Apoptosis

Apoptosis, or programmed cell death, is a growth-limiting regulatory mechanism by which cells can trigger their own demise due to advanced aging in response to extracellular signals or as a result of irreparable cellular or DNA damage. In intrinsic apoptosis, proapoptotic signals cause mitochondrial membrane depolarization. In extrinsic apoptosis, extracellular cues signal through membrane receptors to directly activate the caspase-dependent death cascade. Caspase-independent apoptosis can also occur via the apoptosis-inducing factor (AIF) pathway. Apoptosis also plays an important role in developmental mechanisms, such as preventing the overgrowth of particular neuronal cell lineages in the developing brain and regulating interdigital spacing in limb development.



Mitochondrial signaling and apoptosis. Adapted from Bayir and Kagan, Critical Care (2008) 12:206.

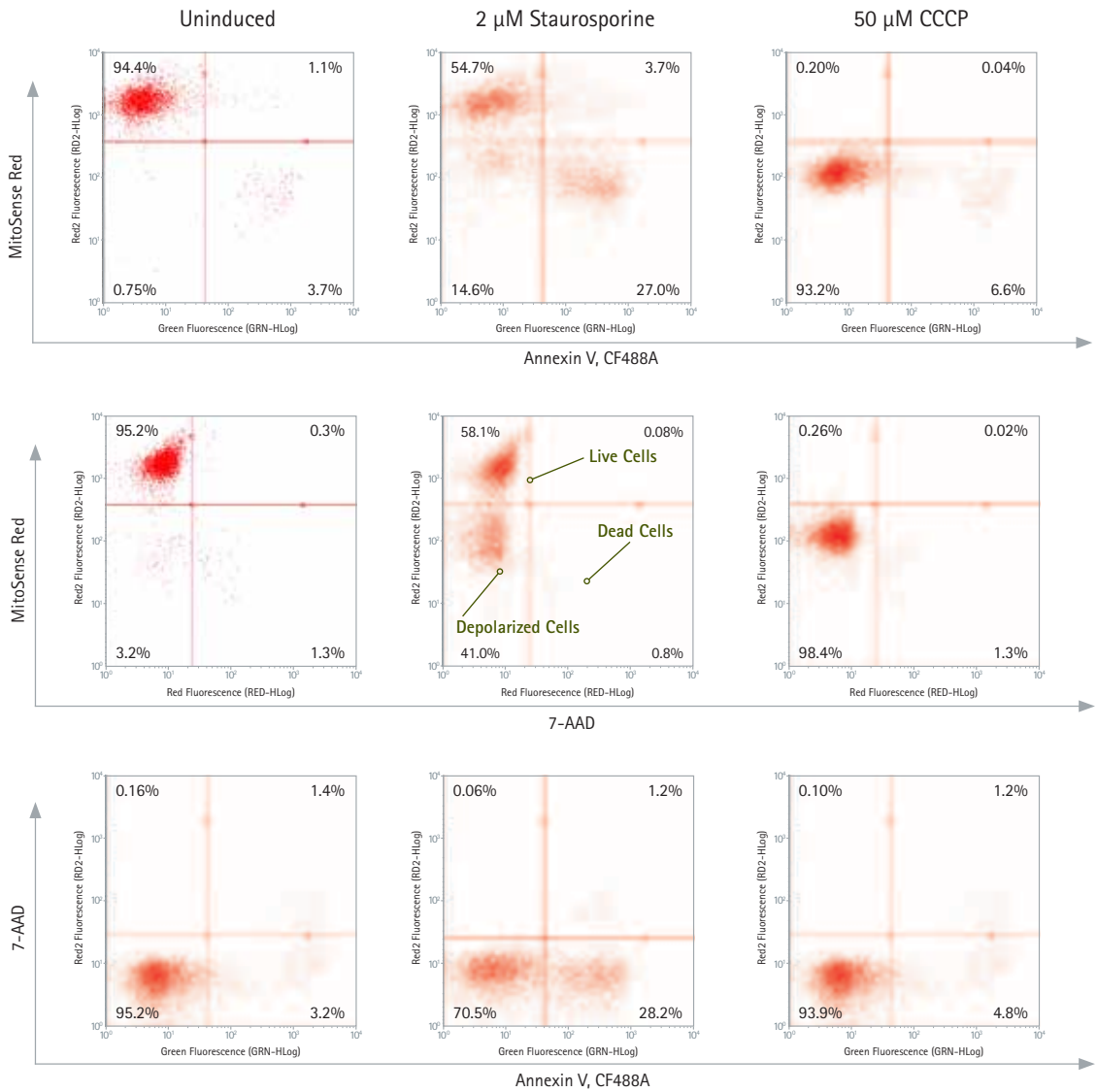
FlowCellect™ MitoDamage Kit

(Catalogue No. FCCH100106)

Mitochondrial health closely correlates with overall cellular health and therefore is an important consideration. For example, stem cells show an increase in mitochondrial potential during successful differentiation. Conversely, reductions in mitochondrial membrane potential in compound screening are often an early indicator of cell death. Harness the power of flow cytometry to assess changes in mitochondrial membrane potential, apoptosis as measured by Annexin V binding, mitochondrial oxidative stress, and cell death, using only minimal cellular samples. The kits shown for cell health may be used with most dual laser flow cytometry systems equipped with a 488 nm and a 640 nm laser.

The FlowCellect™ MitoDamage kit can distinguish multiple populations:

1. Healthy cells with intact mitochondrial membrane
2. Stressed cells with dissipated membrane potential without Annexin V or 7-AAD staining
3. Early apoptotic cells with dissipated membrane potential and Annexin V binding
4. Late apoptotic cells or dead cells with dissipated membrane potentials



Dot plots depicting Jurkat cells stained using the FlowCellect™ MitoDamage kit. Jurkat cells uninduced, induced to apoptosis with 2 µM staurosporine or with 50 µM CCCP, then stained using the MitoDamage kit. Plots show the percentage of positive cells for:

1st row: Apoptosis (Annexin V binding) and mitochondrial membrane potential change

2nd row: Cell death and mitochondrial membrane potential change

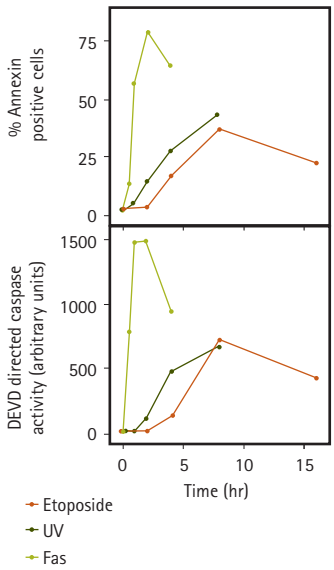
3rd row: Apoptosis and cell death.

Data show that 2 µM staurosporine induces apoptosis in Jurkat cells, and that 50 µM CCCP depolarizes the mitochondrial membrane, but neither condition is sufficient for cell membrane permeabilization and death.

Anti-Fas (human, activating), clone CH11

(Catalogue No. 05-201)

Fas is a transmembrane receptor that activates cell death by binding extracellular Fas ligand. Activated Fas then recruits caspase pathway proteins to its cytoplasmic domain. This monoclonal antibody is ideal for measuring apoptosis and resistance to pro-apoptotic signaling by flow cytometry, immunocytochemistry, or Western blotting. In addition, when this antibody binds to Fas, it activates cell death in multiple cell types. For a complete analysis, use our antibodies to cytoplasmic and mitochondrial death signaling proteins to measure response to pro-apoptotic (Bax, Bak, Bid, Bim) and anti-apoptotic (Bcl-2, Bcl-xl, Bcl-W) signals.

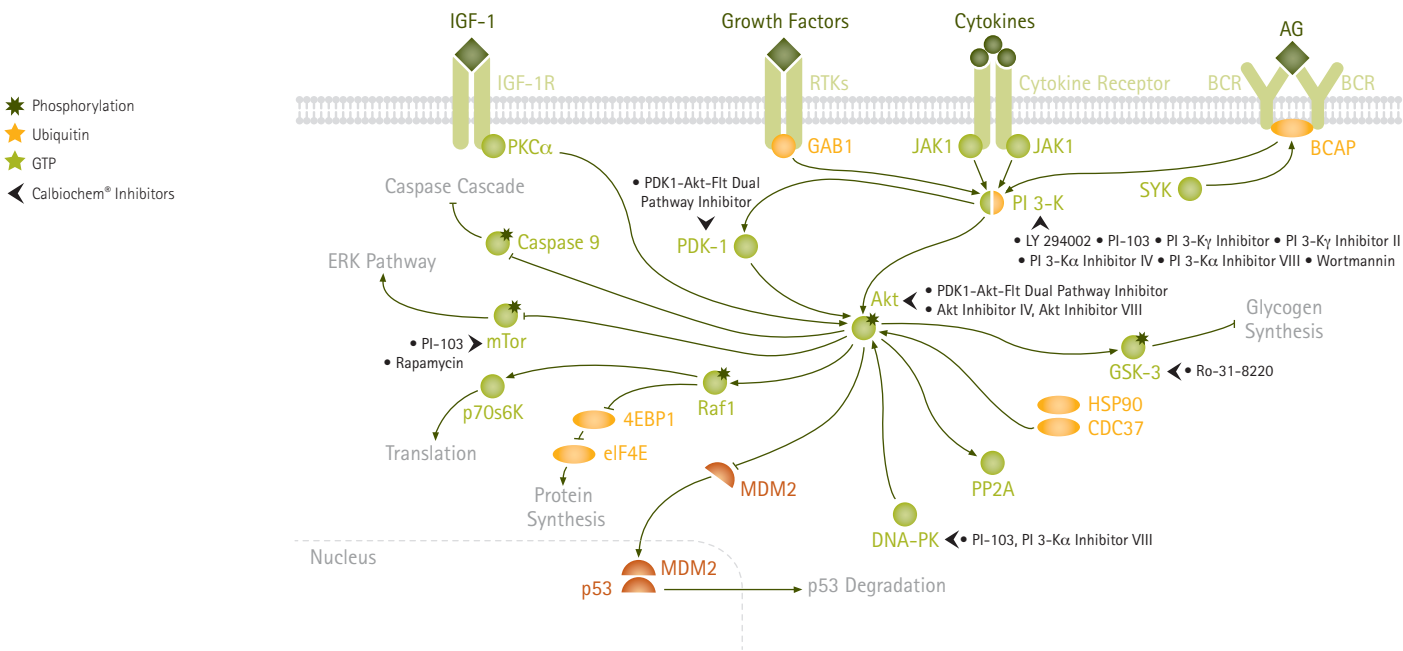


Graphs (left): Etoposide-, UV-, and Fas-mediated apoptosis in Jurkat cells. Apoptotic response, as measured by annexin V binding (top) and caspase activity (bottom), was the fastest upon stimulation with anti-Fas antibody (05-201). This research was originally published in the Journal of Biological Chemistry. Widmann, C et al. 1998; JBC 273(12):7141-7147. © the American Society for Biochemistry and Molecular Biology.

Calbiochem® InhibitorSelect™ Akt/PI 3-K/mTOR Signaling Pathway Inhibitor Panel

(Catalogue No. 124031)

The Akt pathway regulates apoptosis, because Akt can both inactivate pro-apoptotic factors as well as activate transcription of survival genes. This panel of 12 highly potent, selective, and cell-permeable Calbiochem® kinase inhibitors (shown below in black) and a negative control are useful for studying the Akt pathway in one convenient package to help elucidate specific steps in apoptosis.

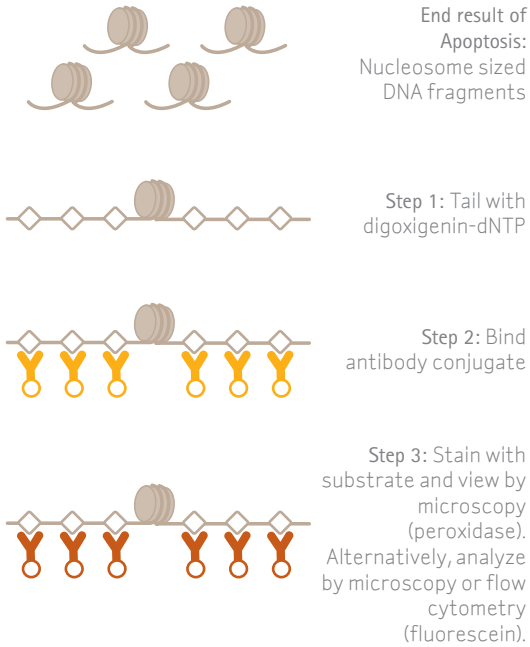


Family of TUNEL Assay Products for Apoptosis Detection

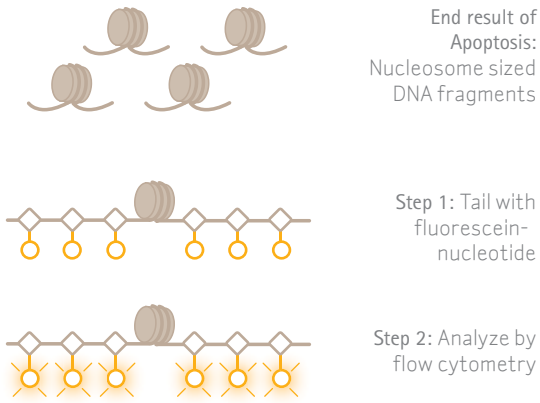
DNA fragmentation is usually associated with ultrastructural changes in cellular morphology in apoptosis. The ApopTag™ family of kits examines apoptosis via DNA fragmentation by the TUNEL assay. The DNA strand breaks are detected by enzymatically labeling the free 3'-OH termini with modified nucleotides. These new DNA ends that are generated upon DNA fragmentation are typically localized in morphologically identifiable nuclei and apoptotic bodies. In contrast, normal or proliferative nuclei, which have relatively insignificant numbers of DNA 3'-OH ends, usually do not stain with the kit. ApopTag™ Kits detect single-stranded and double-stranded breaks associated with apoptosis. Drug-induced DNA damage is not identified by the TUNEL assay unless it is coupled to the apoptotic response. In addition, this technique can detect early-stage apoptosis in systems where chromatin condensation has begun and strand breaks are fewer, even before the nucleus undergoes major morphological changes.

Description	Catalogue No.
ApopTag™ Red *In Situ* Apoptosis Detection Kit	S7165
ApopTag™ Fluorescein Direct *In Situ* Apoptosis Detection Kit	S7160
ApopTag™ Plus Peroxidase *In Situ* Apoptosis Kit	S7101
ApopTag™ Fluorescein In Situ Apoptosis Detection Kit	S7110
ApopTag™ Plus *In Situ* Apoptosis Fluorescein Detection Kit	S7111
ApopTag™ Peroxidase *In Situ* Apoptosis Detection Kit	S7100
ApopTag™ Peroxidase *In Situ* Oligo Ligation (ISOL) Kit	S7200
CaspaTag™ *In Situ* Apoptosis Detection Kits	APT400
CaspaTag™ Caspase 3,7 *In Situ* Assay Kit, Fluorescein	APT403
CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Fluorescein	APT420
CaspaTag™ Caspase 8 *In Situ* Assay Kit, Fluorescein	APT408
CaspaTag™ Caspase 9 *In Situ* Assay Kit, Fluorescein	APT409
CaspaTag™ Caspase 3,7 *In Situ* Assay Kit, Fluorescein	APT423
CaspaTag™ Caspase 8 *In Situ* Assay Kit 25, Fluorescein	APT428
CaspaTag™ Caspase 9 *In Situ* Assay Kit, Fluorescein	APT429
CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Sulforhodamine	APT500
CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Sulforhodamine	APT520
CaspaTag™ Caspase 3,7 *In Situ* Assay Kit, Sulforhodamine	APT523

Indirect



Direct



Key Products

Available from **www.millipore.com**

Antibodies and Proteins

Description	Catalogue No.
Anti-Bak (N-terninus)	06-536
Anti-Bax rabbit monoclonal	04-434
Anti-Bcl-2 rabbit monoclonal	04-436
Anti-Bim, clone 14A8	MAB17001-50UG
Anti-Caspase 3 (proform), clone 3CSP03	MAB4603
Anti-Caspase 7, rabbit monoclonal	04-441
Anti-DR4/TRAIL-R1	06-744
Caspase-3, Human, Recombinant, *E. coli*	235417

Assays

Description	Catalogue No.
ApopNexin® Annexin V FITC Apoptosis Kit	APT750
TUNEL Apoptosis Detection Kit	17-141
MILLIPLEX® MAP Human Apoptosis Panel – 3-Plex	48-670
FlowCellect™ MitoLive Kit	FCCH100107
FlowCellect™ MitoStress Kit	FCCH100109
FlowCellect™ Cytochrome c Kit	FCCH100110
Cytochrome C QCI / HCA Assay Kit	HCS236
FlowCellect™ MitoPotential Red Kit	FCCH100105
FlowCellect™ Annexin Red Kit	FCCH100108
FlowCellect Human B Cell FAS Kit	FCCH100137
FlowCellect™ Human T cell Apoptosis Kit	FCCH100138
FlowCellect™ Human CD4 T cell FAS Kit	FCCH100154
guava® Caspase 9 SR Kit	4500-0500
guava® MultiCaspase FAM Kit	4500-0530

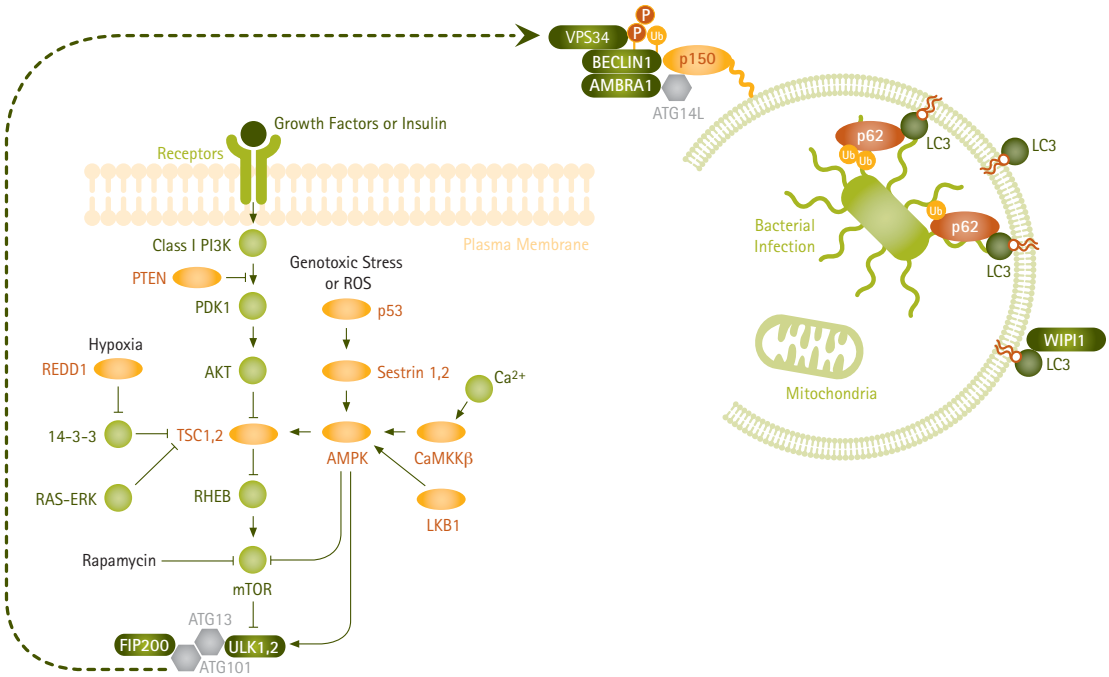
Inhibitors

Description	Catalogue No.
Caspase Inhibitor I	627610
Caspase Inhibitor VI	219007
InSolution™ Q-VD-OPh, Non-O-methylated	551476
Caspase Inhibitor III	218745
Caspase-1 Inhibitor II	400012
Caspase-3 Inhibitor II	264155
Caspase-8 Inhibitor II	218759
DAPK Inhibitor	324788
Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	124018

Autophagy and Protein Recycling

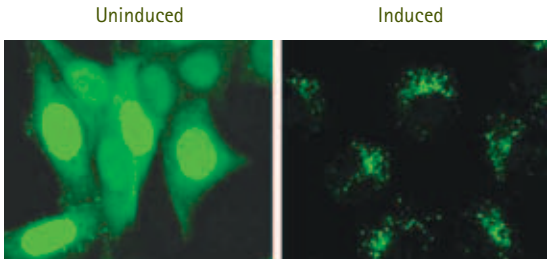
Autophagy is a highly regulated homeostatic degradative process where cells destroy their own components via the lysosomal machinery and recycle them. This process is associated with diverse diseases including Alzheimer's disease, aging, cancers and Crohn's disease. Under extreme conditions of starvation, cells utilize this process to reallocate nutrients from less important processes to essential processes required for survival. However, if cellular damage becomes irreparable, cells can destroy themselves completely by autophagy. Via extensive crosstalk with proapoptotic signaling pathways, autophagy can also contribute to cell death and greatly influence general cell health. Malfunctions of autophagy can influence longevity and productivity of cells to function at full capacity.

Elucidating the correlation between autophagy and apoptotic cell death has become the focus of a great deal of research, particularly in tumor biology. On one hand, autophagy may induce cell death by degrading essential components; on the other hand, it may facilitate survival of cancer cells under unfavorable metabolic conditions. For example, cancer cells with mutated anti-apoptotic Bcl-2 may survive chemotherapy by employing a protective autophagic process.



Types of cellular stress, such as nutrient limitation, hypoxia, oxidative stress, and DNA damage (genotoxic stress), can induce autophagy, either via inhibition of mTOR or by AMPK activation of the ULK1/2 kinase complex (Atg1 in yeast). The ULK1,2/Atg13 complex, by mechanisms still being characterized, prepares cells to construct a double membrane vesicle known as the autophagosome by catalyzing the scaffolding of Atg protein complexes onto the pre-autophagosome (phagophore) membrane. The ATG14L/Vps34 protein complex generates phosphatidylinositol triphosphate, which recruits still more Atg complexes to the membrane. In the final step of autophagy, the outer membrane of autophagosome fuses with the lysosome that provides the hydrolytic enzyme machinery and the contents are degraded and recycled.

Featured Products



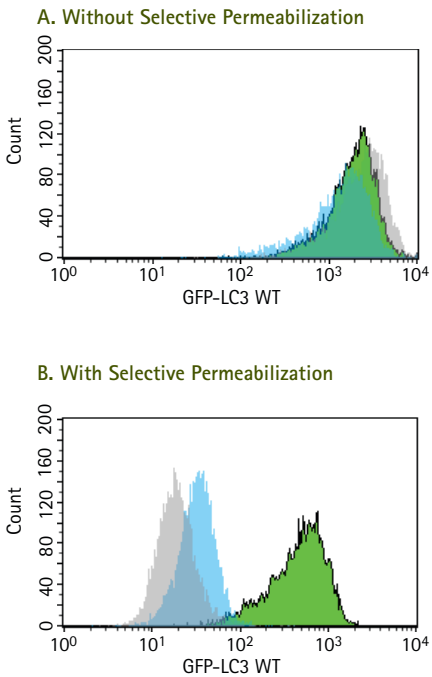
Aggregation of GFP-LC3 in autophagosomes in autophagy-induced cells. HeLa cells were transduced with lentiviral particles. Cells were either left in complete medium (left) or incubated in EBSS with a lysosomal inhibitor (right) to induce autophagy and inhibit lysosomal degradation of autophagosomes.

Watch a video of autophagy occurring in real time by scanning this QR code or by visiting: www.millipore.com/autophagyvideo

LentiBrite™ GFP-LC3 & GFP-LC3 Mutant Lentiviral Biosensors

(Catalogue Nos. 17-10193 & 17-10189)

Visualize autophagy in live cells, in real time with EMD Millipore's LentiBrite™ GFP-LC3 and mutant GFP-LC3 Lentiviral Biosensors. Upon induction of autophagy, LC3 (the mammalian homolog of the small protein Atg8) is conjugated to phosphatidylethanolamine and recruited to the autophagosome membrane, targeting the autophagosome for fusion with the lysosome. These pre-packaged lentiviral particles, encoding normal GFP-LC3 and a GFP-LC3 mutated at the lipdation site, provide bright fluorescence and precise localization of autophagosomal membranes in even difficult-to-transfect cell types, such as stem cells and primary neurons. RFP-LC3 versions will be available as well. Visit our website for our entire family of LentiBrite™ Lentiviral Biosensors.



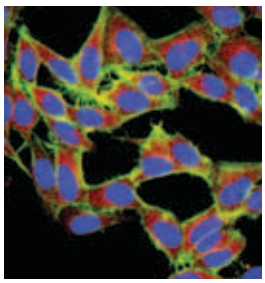
FlowCellect™ LC3-GFP Reporter Autophagy Assay Kits

(Catalogue Nos. FCCH100170 and FCCH100181)

Harness the power of flow cytometry to quantify autophagy and measure potency of autophagy inducers. Included selective permeabilization solution discriminates between cytosolic LC3 from autophagic LC3 by extracting the soluble cytosolic proteins. Monomeric GFP reporter minimizes dimer formation and aggregation, resulting in more accurate analysis. Included autophagy detection reagent prevents lysosomal degradation of LC3, allowing its quantification. Two host cell lines are offered — CHO (Chinese Hamster Ovary) or U20S (human osteosarcoma). The CHO reporter cell line is ideal for flow cytometry applications, while U20S is suitable for both imaging and flow cytometry.

Graphs (left): Flow cytometry detection of LC3 translocation via autophagosomes by addition of a lysosome inhibitor. The FlowCellect™ LC3-GFP Reporter Autophagy Kit was used (A), without selective permeabilization to show no shift of LC3-GFP level before and after starvation (induction of autophagy). The position of the histograms indicates the high level of LC3-GFP expression in the cytoplasm. In (B), with selective permeabilization, LC3-GFP level remains high in autophagosomes when starved in the presence of lysosome inhibitor (green); even without the inhibitor, a slight shift is observed when starved (blue). All the cytosolic LC3-GFP is washed away if no autophagy is induced by starvation (gray).

Confocal immunocytochemistry of HeLa cells using anti-mTOR (Cat. No. 05-1564) shows mTOR (red) expressed in the cytoplasm.



Anti-mTOR, clone 21A12.2

(Catalogue No. 05-1564)

mTOR is a key negative regulator of autophagy, inhibiting the Beclin-1 complex in response to growth factors. Cellular stresses, such as oxidative stress and DNA damage, inhibit mTOR and thus activate autophagy. Validated for Western blotting and immunocytochemistry, anti-mTOR is one of our numerous antibodies for studying autophagy, including antibodies for multiple Atg family proteins.

Key Products

Available from **www.millipore.com**

Antibodies

Description	Catalogue No.
Anti-MAP1LC3B2	AB2970
Anti-MAP1LC3A	AB15412
Anti-MAP1LC3C	AB15414
Anti-UVRAG	AB2960
Anti-SOGA	ABS91
Anti-Beclin-1, clone EPR1733Y	MABN16
Anti-ATG3	AB2953
Anti-ATG4C	ABC21
Anti-ATG5	ABC14
Anti-ATG7	AB10511
Anti-ATG9 L2	AB15407
Anti-ATG10	AB15408
Anti-ATG12	AB15410
Anti-ATG16L1	ABC25
Anti-Akt1/PKBα, clone AW24	04-796
Anti-PKC (α, β, γ), clone M110	05-983
Anti-PI3 Kinase, p110γ, clone 17D7.2	05-1559
Anti-p70 S6 Kinase, clone 20-10C-6	05-781R
Anti-ROCK1	07-1458
Anti-phospho-VEGFR2/Flk-1/KDR (Tyr1223)	07-1294

Assays

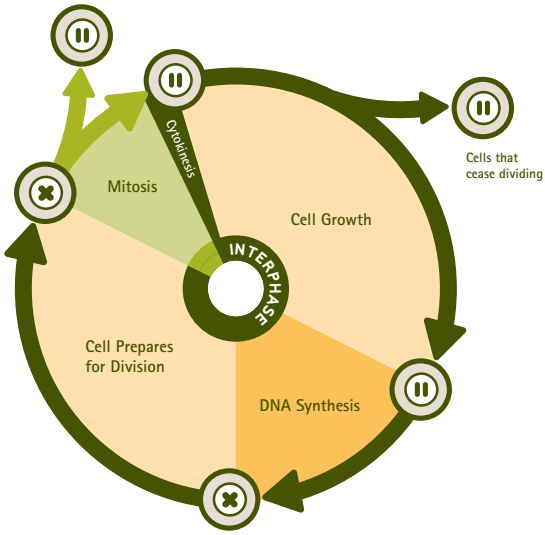
Description	Catalogue No.
MILLIPLEX® MAP Human Akt/mTOR-11plex Panel	48-611

Inhibitors

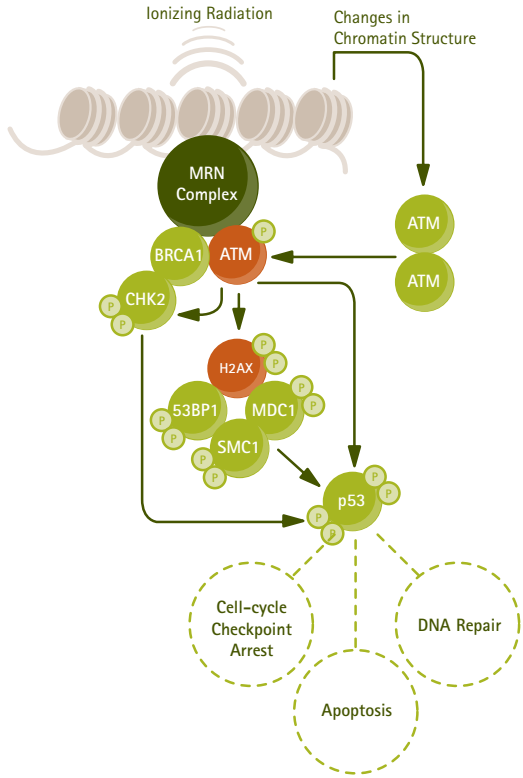
Description	Catalogue No.
Autophagy Inhibitor, 3-MA	189490
Rapamycin	553210
SMER28	573121
mTOR Inhibitor III, PP242	475988

Cell Cycle and DNA Damage

Cell cycle phase distributions can be used to assess cell health, proliferation, as well as the potential mechanism of cytotoxic agents. For example, measuring the population of S phase cells can reflect the amount of newly synthesized DNA. Also, distinguishing cells in G2 from M phase cells can help identify cells undergoing mitosis. Because the cell uses cell cycle checkpoints in order to regulate proliferation when it senses stress signals, there is crosstalk between stress pathways, such as DNA damage, and cell cycle.



Phases of the cell cycle. The cell cycle can be divided into two distinct stages. The first stage is interphase which consists of the G1, S, and G2 phases, in which cells are active, growing, and DNA is being replicated. The second is M phase, also known as the "mitotic phase," in which cell division takes place.



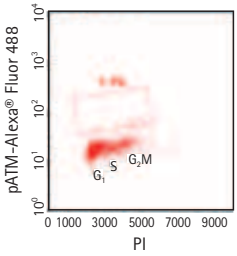
DNA damage signaling pathways. ATM, or Ataxia telangiectasia mutated kinase, is activated in response to double-strand breaks. Once activated, ATM phosphorylates a number of downstream factors, including P53, CHK2, SMC1, NBS1, and Histone H2A.X, inducing DNA repair, cell cycle arrest, apoptosis, or autophagy.

FlowCellect™ Cell Cycle Checkpoint ATM Kit

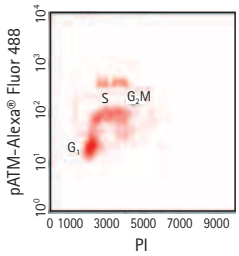
(Catalogue No. FCCH025143)

Easily identify, discriminate, and quantitate cells undergoing DNA damage relative to positioning within the cell cycle, using the power of flow cytometry. This kit includes a directly conjugated Anti-phospho-ATM (Ser1981) Alexa Fluor® 488 conjugate plus a DNA dye (Propidium Iodide) to identify where DNA damage takes place within the cell cycle. The kit includes an optimized protocol with all of the necessary components to provide a true "plug and play" assay for DNA damage and cell cycle checkpoints.

A. Untreated



B. Topotecan-treated

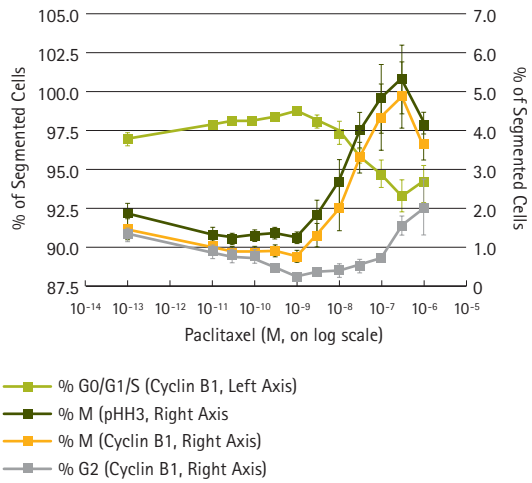


Bivariate analysis detecting DNA damage induced by Topotecan in relation to cell cycle positioning: Topotecan is a topoisomerase1 inhibitor which causes DSBs in the late G1 to early S phases in the cell cycle, maximal at mid S phase. Treated HeLa cells show a marked increase in ATM phosphorylation, indicating DNA damage (B), when compared to the untreated sample (A), 33% versus 1%, respectively.

Phospho-Histone H3 Ser10 and Cyclin B1 Assay

(Catalogue No. HCS211)

Histone H3 plays an important regulatory role in cell proliferation while cyclin B1 is a key protein in triggering mitosis. High Content Screening (HCS) of histone H3 and cyclin B1 enable distinction among G2, M, and G0/G1/S phases of the cell cycle and has been used as a readout in cell cycle inhibitor profiling studies.



Cell cycle phase analysis of HeLa cells treated for 4 hours with serial dilutions of paclitaxel. Cell handling, fixation and immunostaining were performed according to the protocol for HCS211.

Key Products

Available from **www.millipore.com**

Antibodies and Proteins

Description	Catalogue No.
Cell Cycle-G2/M Phase Pathway Explorer Antibody Minipack	15-120
Anti-Wee1	06-972
Anti-Cdk5, clone DC17	05-364
Anti-Cdc42-interacting protein 4	ABS69
TGFBR-1, active	14-912

Assays

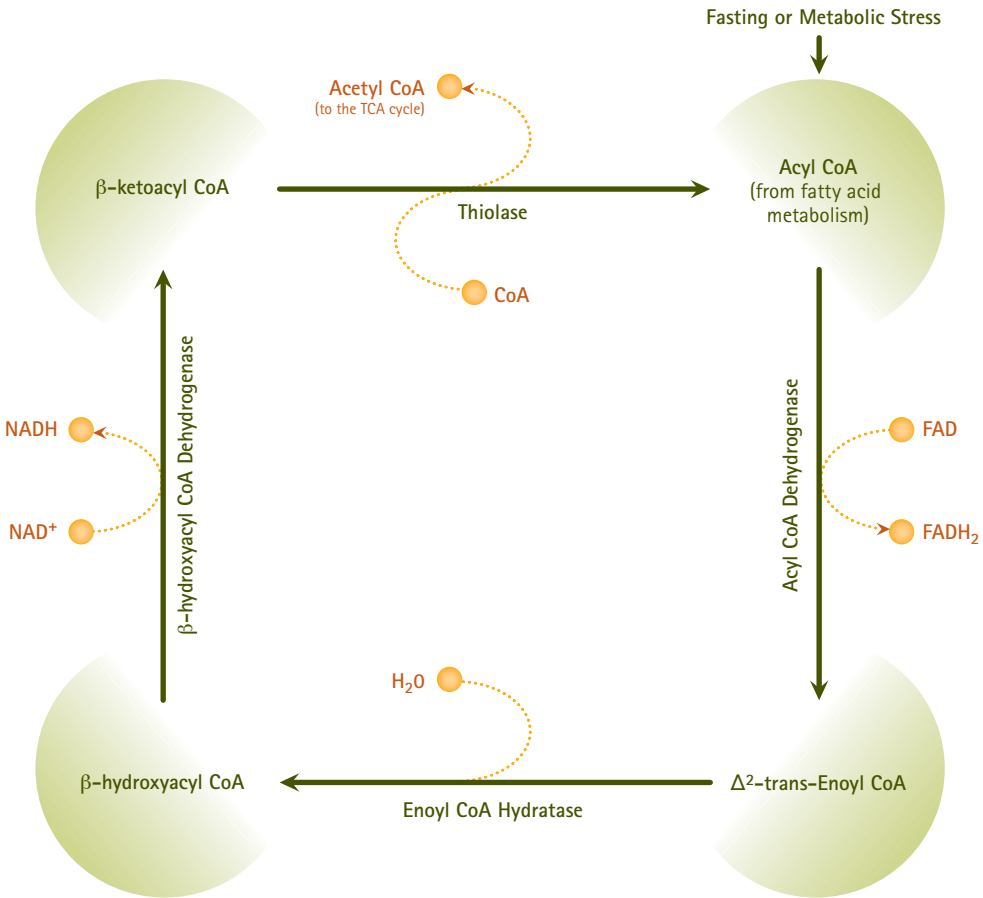
Description	Catalogue No.
Cyclin B1 and Ki-67 QCI/HCA Assay Kit	HCS210
FlowCellect™ Bivariate Cell Cycle Kit for DNA Replication Analysis	FCCH025102
FlowCellect™ Bivariate Cell Cycle Kit for G2/M Analysis	FCCH025103
FlowCellect™ DNA Damage (H2A.X) and Cell Cycle Analysis Kit	FCCH025142
FlowCellect™ Multicolor DNA Damage Response Kit	FCCH025104
FlowCellect™ H2A.X DNA Damage Dual Detection Kit	FCCS025153
FlowCellect™ Histone H2A.X Phosphorylation Assay Kit	FCCS100182
guava® Cell Cycle Reagent Propidium Iodide Solution	4500-0220
MILLIPLEX® MAP Human Multi-Pathway Signaling Kit , Phosphoprotein	48-680
MILLIPLEX® MAP TGFβ 3-Plex	TGFB-64K-03

Inhibitors

Description	Catalogue No.
ATM Kinase Inhibitor	118500
ATM/ATR Kinase Inhibitor	118501

Metabolic and Endoplasmic Reticulum (ER) Stress

Cellular metabolism is the set of pathways by which cells produce energy from organic substances. Accordingly, metabolic stress occurs when there is an imbalance in these pathways, resulting in cellular stress response. One modulator of metabolic stress is ER stress, caused by prolonged unfolded protein response (UPR) in the endoplasmic reticulum. Because a highly oxidative environment is required in the ER for proper folding of proteins before they are secreted, unfavorable redox conditions can contribute to the accumulation of unfolded proteins in the ER, eliciting the UPR. The UPR helps the ER to adapt to its changed environment and re-establish normal ER function by upregulating genes that enhance the protein-folding capacity of the ER and promoting protein degradation to remove misfolded proteins. Prolonged stress on ER leads to apoptosis. Studying metabolic and ER stress requires monitoring of key biomarkers in the mitochondria (energy factories of the cell) as well as ER and UPR signaling pathway proteins.

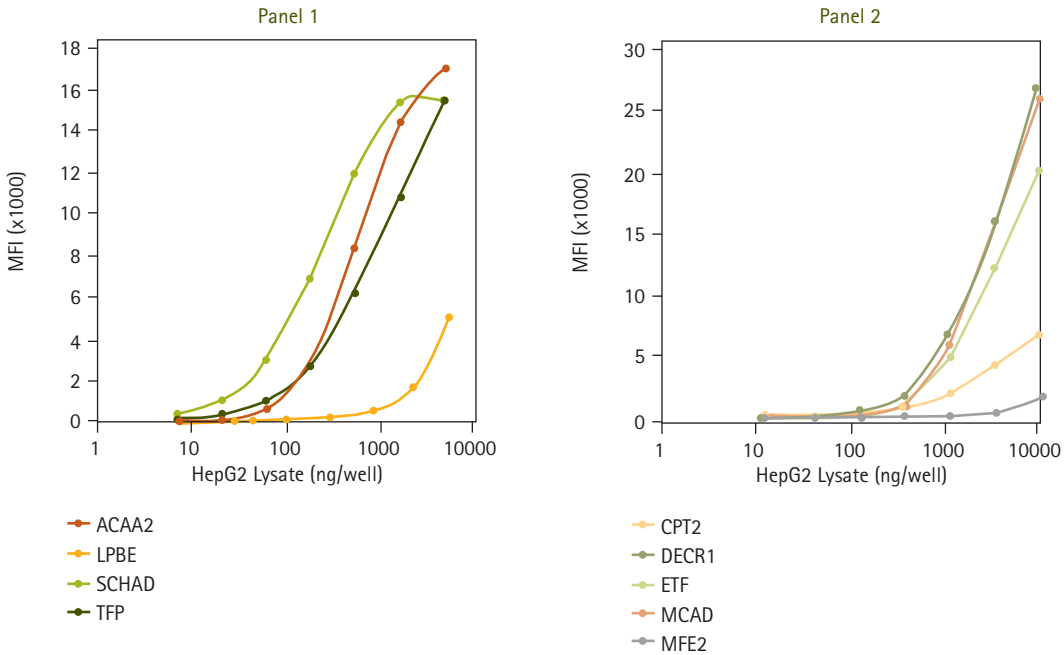


Key steps of the β-oxidation pathway used to metabolize fatty acids in order to generate energy during periods of fasting and metabolic stress. Long-chain, medium-chain or short chain fatty acids enter the pathway as acyl CoA. Each β-oxidation cycle yields one molecule of acetyl CoA which enters the TCA energy production cycle.

MILLIPLEX® MAP Human Fatty Acid Oxidation Magnetic Bead Panels 1 & 2

(Catalogue Nos. HFA01MAG-11K and HFA02MAG-11K)

The FAO pathway, located in the mitochondria and peroxisomes, is composed of more than 25 enzymes and transport proteins that regulate the degradation of fatty acids. Monitoring the FAO pathway (especially the β-oxidation pathway) and any potential cellular metabolism changes in the human tissues and cells can reveal mechanisms of response to disease states, drug treatments, dietary changes or genetic mutations. The MILLIPLEX® MAP Human Fatty Acid Oxidation Panel 1 includes key enzymes ACAA2, LPBE, SCHAD, and TFP; the MILLIPLEX® MAP Human Fatty Acid Oxidation Panel 2 includes key enzymes CPT2, DECR1, ETF, MCAD, and MFE2. These enzymes are involved in the β-oxidation pathway, and quantification of these targets simultaneously in one reaction well provides a more accurate snapshot of pathway activity.

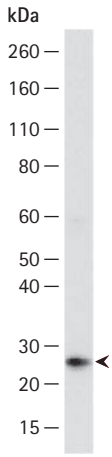


Multiplex analysis of HepG2 cell lysates with the MILLIPLEX® MAP Human Fatty Acid Oxidation Panel 1 (Left, Catalogue No. HFA01MAG-11K) and Panel 2 (Right, Catalogue No. HFA02MAG-11K). Refer to the product protocol for examples of use with other human cell lysates and tissues.

Anti-XBP1

(Catalogue No. 09-722)

X-box Protein 1 (XBP1) is a transcription factor that belongs to the bZIP family. XBP1 plays an important role in the functioning of the immune system and in the ER stress response. It is essential for the differentiation of plasma cells and is involved in the regulation of the MHC class II genes. XBP1 helps initiate the ER stress response by binding to the unfolded protein response element (UPRE) which activates UPR target genes. Mutations in XBP1 have been associated with mental disorders.

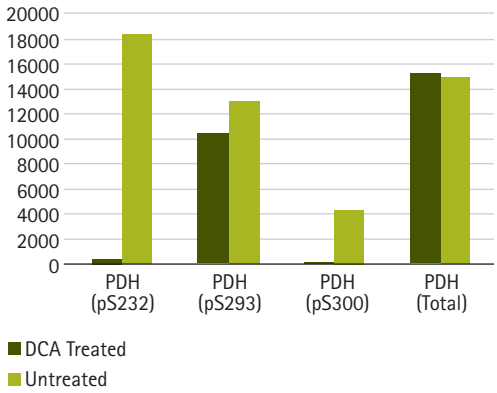


Blot (right): Anti-xBP1 protein recognizes XBP1 in LnCAP cells as shown by Western blotting. LNCAP cell lysate was resolved by electrophoresis, transferred to PVDF membranes and probed with Anti-XBP1 (1:125) dilution. Proteins were visualized using a Donkey anti-Rabbit IgG conjugated to HRP using a chemiluminescence detection system.
Arrow indicates XBP1 (~29 kDa).

MILLIPLEX® MAP Multispecies Pyruvate Dehydrogenase (PDH) Complex Magnetic Bead Panel

(Catalogue No. PDHMAG-13K)

The PDH complex regulates cellular metabolism, and has roles in cancer, neurodegeneration, cardiovascular disease and diabetes. PDH complex activity influences the shift between glycolytic energy production and the Krebs cycle and subsequent oxidative phosphorylation, and thereby reverses the aerobic glycolysis (also known as the Warburg effect) that is a feature of many cancers. This assay kit enables the multiplex detection of the phosphorylation state of multiple sites on PDH to assess its activation status.



Graph (right): Quickly quantify differences in multiple biomarkers in each sample using the MILLIPLEX® MAP Multispecies PDH Complex Panel (Catalogue No. PDHMAG-13K). HepG2 cells treated with DCA (dichloroacetic acid), a PDK inhibitor, show physiologically relevant reduction in PDH phosphorylation at Serines 232, 293 and 300, while the total level of PDH complex remained relatively unchanged.

Calbiochem® PhosphoDetect™ Anti-PDH-E1α (pSer293) Rabbit pAb

(Catalogue No. AP1062-50UG)

Visualize phosphorylation of the PDH complex (PDC) using PhosphoDetect™ antibodies against phosphorylated PDH. The activity of PDC is mainly regulated by the phosphorylation state of Ser293, Ser232, and Ser300 on the E1α subunit. This antibody is thoroughly validated for Western blot, immunocytochemistry and immunoprecipitation.

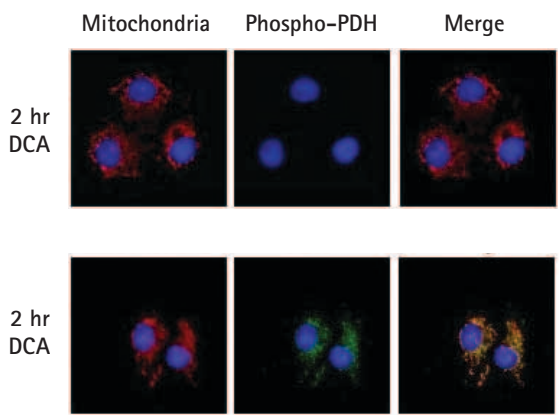


Figure (right): Detection of phospho-PDH-E1α (Ser293) by immunocytochemistry. COS7 cells were incubated in dichloroacetate (DCA), an inhibitor of PDH kinase. All samples were incubated with mitochondrial stain (red), fixed and permeabilized. Primary antibodies: PhosphoDetect™ Anti-PDH-E1α (pSer293) Rabbit pAb (Cat. No. AP1062, green, top) or anti-PDH-E1α antibody (green, bottom). Detection: fluorescence (Alexa Fluor 488 secondary antibody) with DAPI (blue). Data courtesy of Sandra Wiley and Matthew Rardin, University of California, San Diego.

Key Products

Available from **www.millipore.com**

Antibodies and Proteins

Description	Catalogue No.
Anti-ATF6	09-069
Anti-SYVN1	ST1623-100UG
Anti-AMPKα1	07-350
PERK, GST-Fusion, Human, Recombinant, *E. coli*	324881-5UG

Assays

Description	Catalogue No.
MILLIPLEX® MAP Phospho GSK3β (Ser9) MAPmate	46-690
MILLIPLEX® MAP Total GSK3β MAPmate	46-689
GSK-3β ELISA Kit	CBA068 ■
Mitochondrial Complex I Activity Assay Kit	AAMT001-1KIT ■
Mitochondrial Complex II Activity Assay Kit	AAMT002-1KIT ■
Mitochondrial Complex IV (Human) Activity Assay Kit	AAMT004-1KIT ■
Mitochondrial Complex V (ATP synthase) Activity Assay Kit	AAMT005-1KIT ■
Mitochondrial Complex IV (Mouse) Activity Assay Kit	AAMT006-1KIT ■
PDH Activity Assay Kit	AAMT008-1KIT ■
Aconitase Activity Assay Kit	AAMT009-1KIT ■
Cytochrome C ELISA kit	EAMT001-1KIT ■
Frataxin ELISA Kit	EAMT002-1KIT ■
PDH ELISA Kit	EAMT003-1KIT ■

Inhibitors

Description	Catalogue No.
Hexokinase II Inhibitor II, 3-BP	376817-100MG
Glycogen Synthase Kinase (GSK)3β Inhibitor I	361540
Insolution™ GSK-3 Inhibitor IX	361552

■ Available from **www.emdbiosciences.com**

Inflammation and Tissue Damage

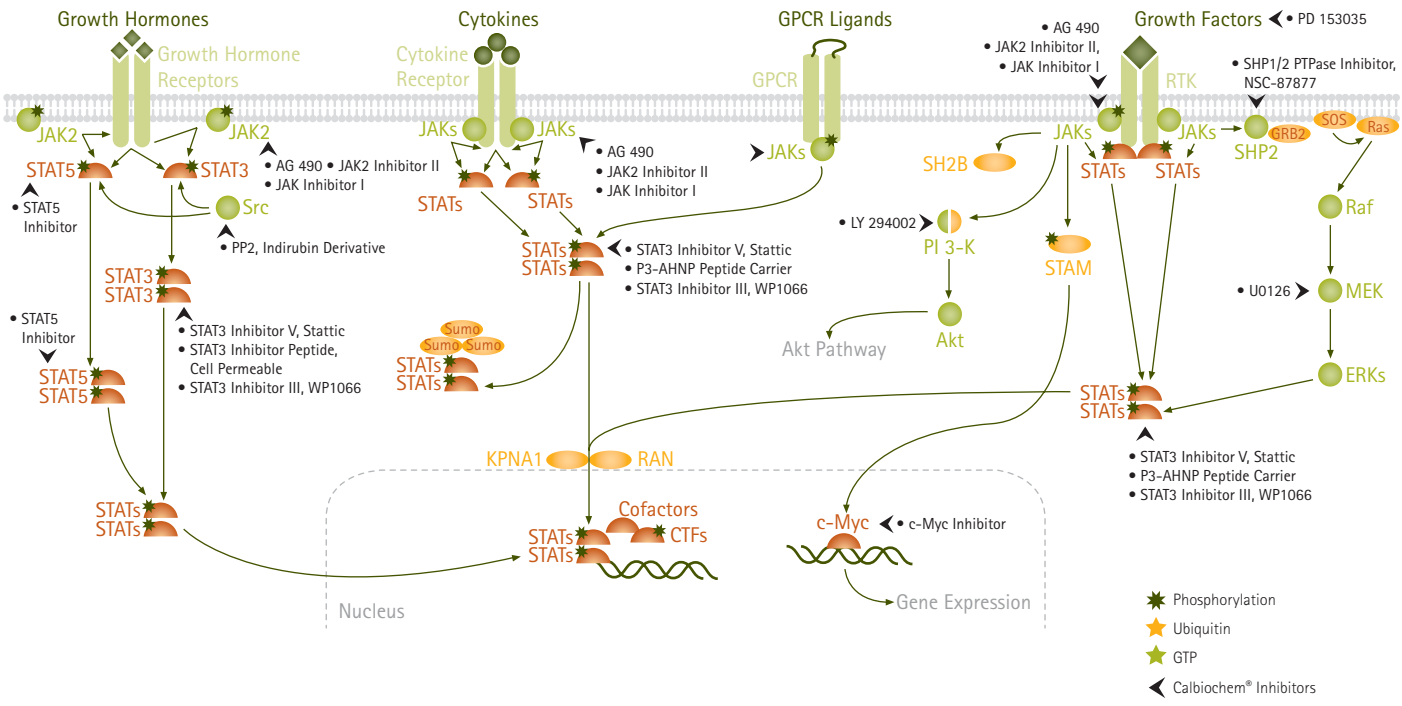
Cellular responses to acute and prolonged inflammation and tissue damage are inextricably linked to the immune response via cytokine/chemokine signaling. Inflammation response has also been associated with susceptibility to diabetes, autoimmune disease, and neurodegeneration, making inflammation and tissue damage subjects of intensive research.

Featured Products

Calbiochem® InhibitorSelect™ JAK/STAT Signaling Pathway Inhibitor Panel

(Catalogue No. 420138)

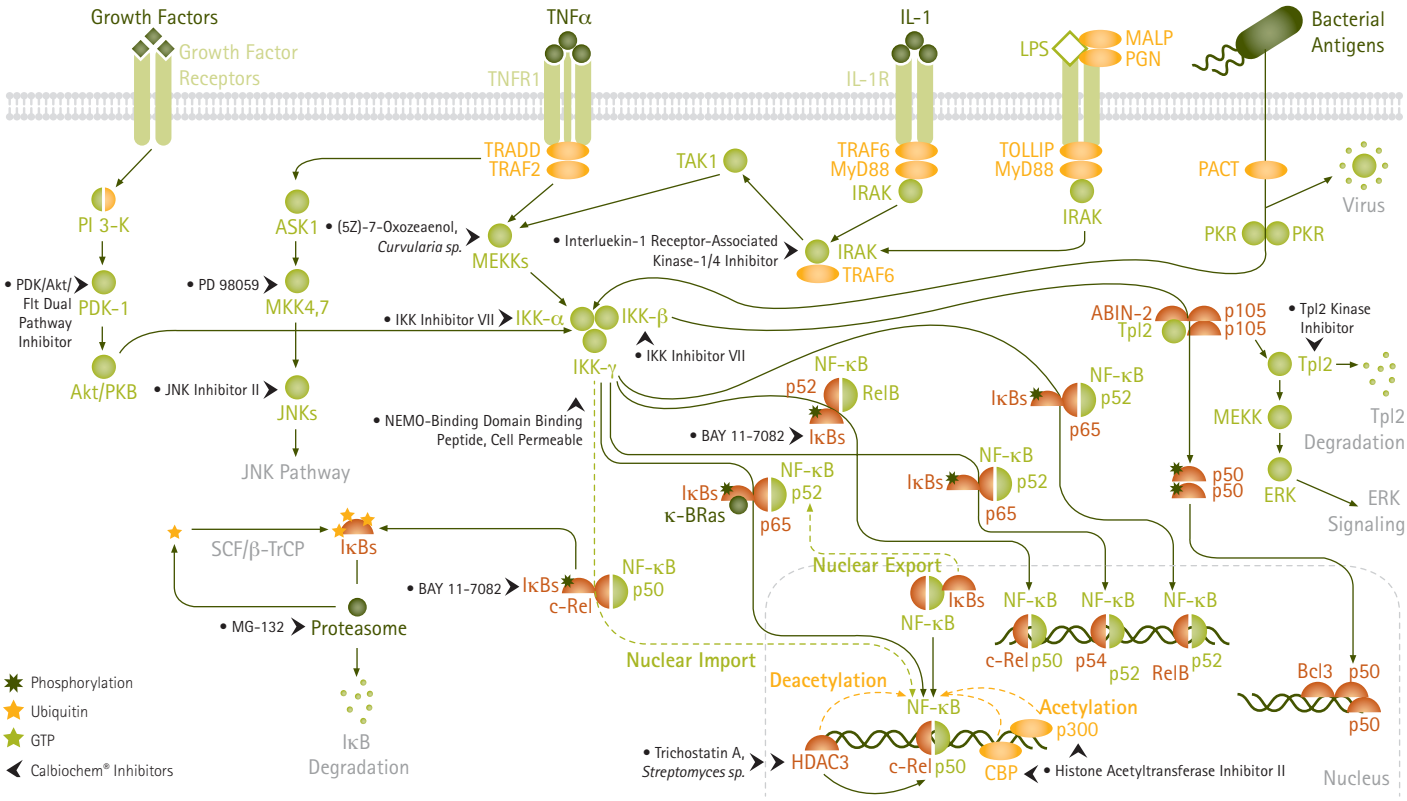
The Janus kinase (JAK) signal transducers and activators of transcription (STAT) play an important role in cell proliferation, cell differentiation, cell migration, and cell death. The JAK/STAT signaling pathway is the principal signaling mechanism for a variety of cytokines and growth factors. Constitutive activation or dysregulation of JAK/STAT signaling can result in inflammatory disease, erythrocytosis, gigantism, and leukemia. This panel of 13 highly potent, selective, and cell-permeable inhibitors (shown below in black) and a negative control is ideal for the investigation of the JAK/STAT signaling pathway.



Calbiochem® InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel

(Catalogue No. 481487)

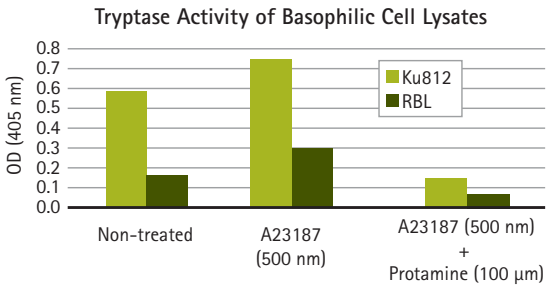
The eukaryotic nuclear factor κB (NF-κB) plays an important role in inflammation, autoimmune response, cell proliferation, and apoptosis by regulating the expression of genes involved in these processes. This panel of 14 highly potent, selective, and cell-permeable inhibitors (shown below in black) and a negative control is ideal for investigating NF-κB signaling pathways.



Mast Cell Degranulation Assay Kit

(Catalogue No. IMM001)

Mast cells are known to accumulate at sites of inflammation, such as asthma, allergic rhinitis, and rheumatoid arthritis. During the inflammatory response, mast cells secrete cytoplasmic granules containing tryptase, suggesting this enzyme has the potential to be used as marker for mast cell activation and inflammation. This kit provides a quick, efficient and sensitive system for evaluation of tryptase activity in cell lysates, supernatants or for inhibitor screening.

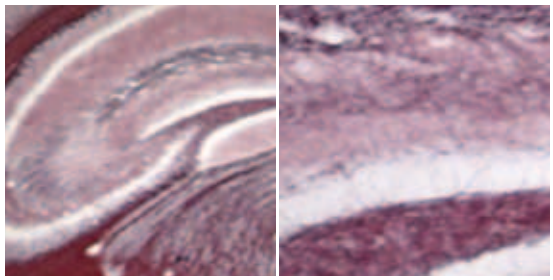


Graph (left): Tryptase Activity of Basophilic Cell Lysates. Human KU812 and Rat RBL basophils were treated with Calcium Ionophore A23187 (+/- Protamine). After the 1-hour incubation period at 37°C, the cells were lysed and tryptase activity was determined.

BLACK-GOLD® II Stain

(Catalogue No. AG400)

BLACK-GOLD® II Stain is ideal for studies of tissue damage in the nervous system, as it can be used to localize both normal and pathological myelin. It can be used to analyze tissues damaged by Multiple Sclerosis (MS), caused by the autoimmune destruction of the myelin layer surrounding neurons in the brain and spinal cord. When the myelin is damaged, nerve messages are sent more slowly and less efficiently. Patches of scar tissue, called plaques, form over the affected areas, disrupting nerve communication. BLACK-GOLD® II stains large myelinated tracts dark red-brown, while myelinated fibers appear black.



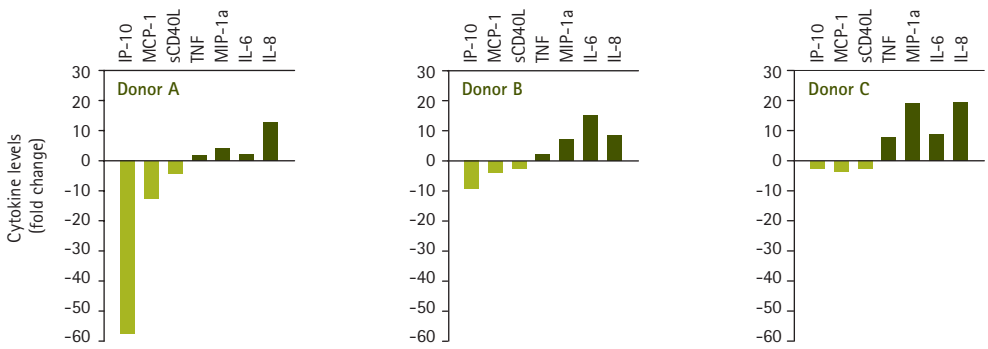
BLACK-GOLD® II staining of cryosectioned mouse brain tissue. Low power magnification (5X) of hippocampus, thalamus, and part of the sensory motor cortex (left). 20X view of the hippocampus (right).

MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel I

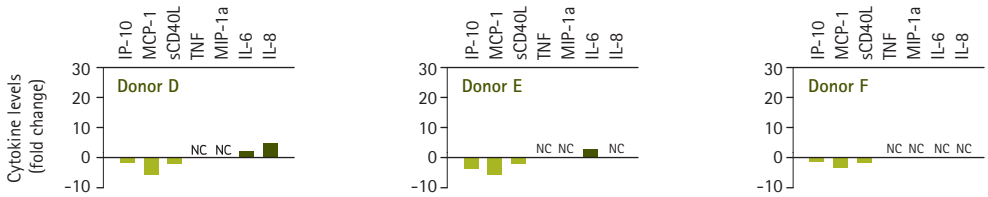
(Catalogue No. HCYTOMAG-60K)

This MILLIPLEX® MAP multiplex analyte panel enables simultaneous characterization of multiple biomarkers associated with individual responses to inflammation and immune signaling, increasing the sensitivity and specificity of the assay while conserving precious samples.

Group A



Group B



In a clinical trial for the anticancer drug NSC 640488, researchers used the MILLIPLEX® MAP Human Cytokine 42-plex assay (Catalogue No. HCYTOMAG-60K) to identify a consistent pattern of cytokine/chemokine modulation in the peripheral blood lymphocytes (PBL) of human donors that responded best to NSC 640488. Shown are fold changes in cytokine concentrations in PBLs from subjects treated with the drug as compared to untreated PBLs from different donors. A small cohort of donors showed downregulation of IP-10, MCP-1, and sCD40L and increased induction of TNFα, MIP-1α, IL-6, and IL-8. This pattern of detection may potentially correlate with a patient's responsiveness to NSC 640488: high responders (group A) or low responders (group B). NC denotes less than a 1.5-fold change.

Key Products

Available from **www.millipore.com**

Antibodies

Description	Catalogue No.
Anti-IL-6, clone 1G3	MABF41
Anti-Mast Cell Tryptase	444905
Anti-phospho-STAT1	07-714
Anti-JAK2	06-255

Assays

Description	Catalogue No.
MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel II	HCYP2MAG-62K
MILLIPLEX® MAP Mouse Cytokine/Chemokine Magnetic Bead Panel I	MCYTOMAG-70K
MILLIPLEX® MAP Non-Human Primate Cytokine Magnetic Bead Panel	PRCYTOMAG-40K
MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel III	HCYP3MAG-63K
MILLIPLEX® MAP Human High Sensitivity Cytokine/Chemokine Magnetic Bead Panel	HSCYTMAG-60SK
MILLIPLEX® MAP Mouse Cytokine/Chemokine Magnetic Bead Panel II	MCYP2MAG-73K
MILLIPLEX® MAP Mouse Cytokine/Chemokine Magnetic Bead Panel III	MCYP3MAG-74K
MILLIPLEX® MAP Rat Cytokine/Chemokine Magnetic Bead Panel	RCYTOMAG-80K
MILLIPLEX® MAP Canine Cytokine/Chemokine Magnetic Bead Panel	CCYTOMAG-90K
MILLIPLEX® MAP Mouse Acute Phase Magnetic Bead Panel 1	MAP1MAG-76K
MILLIPLEX® MAP Mouse Acute Phase Magnetic Bead Panel 2	MAP2MAG-76K
Phosphorylated Neurofilament (pNF-H) Sandwich ELISA Kit	NS170
GFAP ELISA Kit	NS830

Inhibitors

Description	Catalogue No.
Ibuprofen (+/-)	401003
Indomethacin	405268
Celastrol	219465

Oxidative Stress and Hypoxia

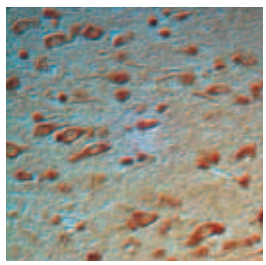
Oxidative stress is characterized by an excess of free radical groups, which creates a potentially unstable cellular environment linked to tissue damage, accelerated aging and degenerative disease. Oxidative stress can result from many factors, including exposure to alcohol, medications, poor nutrition, trauma, cold, hypoxia/reoxygenation, toxins and overexercise. Mitochondria are critical cellular organelles that produce 90% of cellular energy, control cell survival by regulating apoptosis, and produce free radicals called reactive oxygen species (ROS) in response to hypoxia/reoxygenation. Mitochondrial ROS generation results in oxidative stress, damage and cell death by apoptosis or to cellular energetic decline. Therefore, mitochondrial dysfunction caused by disease or compound treatment has dire consequences that can result in cell death. Monitoring impact on mitochondria and related cell health markers is an important part of disease research, apoptosis studies, and mapping pathways of oxidative stress and hypoxic response.

Featured Products

Anti-8-Hydroxydeoxyguanosine (8OHdG)

(Catalogue No. AB5830)

8-Hydroxydeoxyguanosine (8OHdG) is a modified base that occurs in DNA due to attack by hydroxyl radicals that are formed as byproducts and intermediates of aerobic metabolism and during oxidative stress. EMD Millipore's anti-8 hydroxydeoxyguanosine has been shown by ELISA to be completely specific for oxidized DNA while not cross-reacting with other naturally occurring nucleotides. This antibody is a valuable tool for elucidating the role of free radical damage in a number of human disease states.

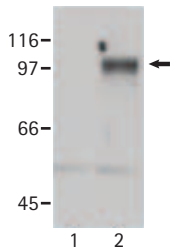


Goat anti-8-OHdG (Catalogue No. AB5830) Alzheimer disease brain showing immunohistochemcal staining of oxidized DNA in neurons.

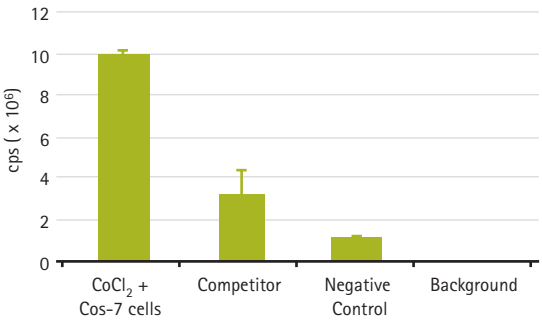
Anti-HIF-1α

(Catalogue No. 07-628)

HIF-1 (Hypoxia Inducible Factor-1) is one of the key regulators of the transcriptional response to oxygen deprivation. HIF-1 is composed of two subunits, HIF-1α and HIF-1β also known as aryl hydrocarbon receptor nuclear translocator (ARNT) that are members of the basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) family of transcription factors. HIF-1 is essential for angiogenesis, embryonic development, and is associated with tumor progression, erythropoiesis, vascular development/remodeling, vasodilation, and glucose/energy metabolism.



Modified nuclear extract from normal MCF-7 cells (Lane 1) and MCF-7 cells treated with 150 mM cobalt chloride (a hypoxia mimetic) for 16 hours (Lane 2) and analyzed by Western blotting with anti-HIF-1α (07-628).



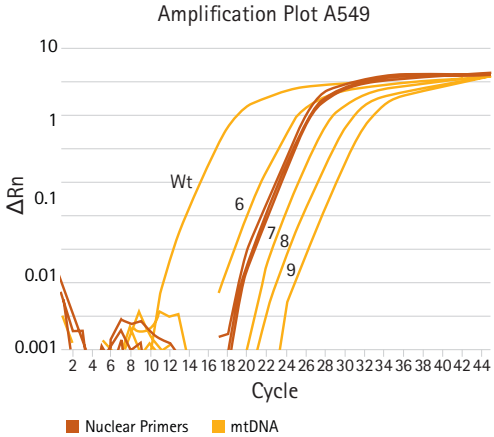
HIF-1α EZ-TFA Transcription Factor Assays

(Catalogue Nos. 70-670, 70-570, 70-500)

The transcription factor HIF-1α is a key regulator of the hypoxia response, mediating both metabolic reprogramming of tumor cells as well as stimulating angiogenesis. EMD Millipore's HIF-1α EZ-TFA Transcription Factor Assays are powerful tools for measuring the DNA binding activity of HIF-1α in nuclear extracts. The assays are provided in non-radioactive, 96-well formats.

Graph (left): Nuclear extract from $CoCl_2$-treated Cos-7 cells were assayed with the EZ-TFA HIF-1α assay kit and showed robust binding to the DNA capture probe, which is complementary to the endogenous HIF-1α DNA-binding sequence, the hypoxia response element (HRE). Specific binding was confirmed, as addition of specific competitor oligonucleotide reduced the observed signal. Signal was further diminished in the absence of DNA capture probe ("Negative Control").

Technology Highlight



Ethidium Bromide (EtBr)-treated A549 Human Lung Epithelial Carcinoma Cells were cultured in 50 ng/mL EtBr (passage numbers shown next to curves, in black). EtBr is concentrated differentially in the mitochondria due to higher mitochondrial membrane potential and subsequent DNA binding. Cells were directly lysed in PCR reactions, total DNA normalized to 1 ng/mL and targets amplified using paired mitochondrial (mtDNA) or nuclear primers in a NovaQUANT™ qPCR assay with SYBR® Green technology. Higher passage numbers lead to a greater depletion of mtDNA as cells transition to a glycolytic energy state. Dark lines show no change in nuclear DNA. Wt equals wildtype.

NovaQUANT™ Quantitative PCR (qPCR) Assays for Mitochondrial Research

Step ahead of the field: accelerate your mitochondrial research.

Mitochondrial research is an expanding field with few standardized experimental tools. NovaQUANT™ qPCR assays are an innovative, reliable and user-friendly way to study mitochondrial function by detecting expression of mitochondrial oxidative phosphorylation and oxidative stress genes and quantifying mitochondrial-to-nuclear DNA ratios. All kits contain optimized primer pairs for sensitive, specific qPCR.

Available from **www.emdbiosciences.com**

NovaQUANT™ Human and Mouse Mitochondrial to Nuclear DNA Ratio Assay

(Catalogue Nos. 72620 and 72621)

This qPCR assay for accurate detection of mitochondrial (mt) and nuclear DNA in cells or tissues is the first standardized assay for mitochondrial research. Ratio of mitochondrial to nuclear DNA is a key marker for assessing cellular health affected by cell differentiation, stress, disease, exercise, caloric intake, and toxicity. Optimized protocols and convenient, pre-aliquoted primers make determination of cell health easy for any laboratory.

NovaQUANT™ Mitochondrial Biogenesis qPCR Assay

(Catalogue Nos. 72625 and 72626)

Mitochondrial biogenesis is activated by numerous stimuli, including cellular stress, and is important to researchers studying cell survival or injury repair. This qPCR kit contains pre-aliquoted optimized primer pairs targeting 12 oxidative phosphorylation genes, including one master regulator and three housekeeping/control genes. The kit is widely applicable to oxidative stress studies, and is optimized for sensitive and specific profiling of mRNA levels in cells undergoing mitochondrial biogenesis.

NovaQUANT™ Mitochondrial Oxidative Stress qPCR Assay

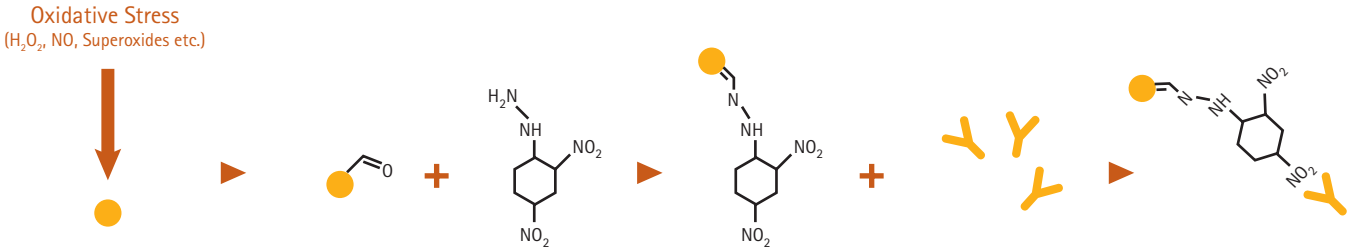
(Catalogue Nos. 72627 and 72628)

Mitochondria are involved in production of reactive oxygen species associated with many diseases including cardiovascular or neurodegenerative disorders. Oxidative stress can result in damage to cellular structures leading to cell death by apoptosis or reduced energetic metabolism and aging. This optimized qPCR kit uniquely detects changes in mRNA levels associated with 20 key oxidative stress genes, one master regulator and three housekeeping/control genes, and is widely applicable to oxidative stress studies.

Technology Highlight

Oxidative Stress Detection with OxyBlot™, ELISA, IC, IH, & flow cytometry

Oxidative modification of proteins by oxygen free radicals and other reactive species such as hydroxynonenal occurs in physiologic and pathologic processes. As a consequence of the modification, carbonyl groups are introduced into protein side chains by a site-specific mechanism. EMD Millipore's oxidative stress detection kits enable simple and sensitive immunodetection of these carbonyl groups.

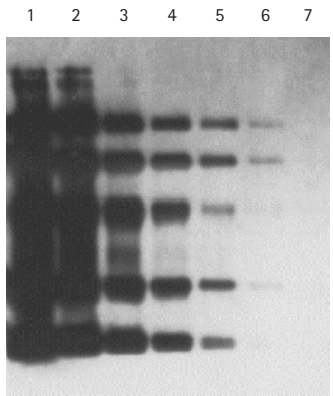


OxyBlot™ Protein Oxidation Detection Kit

(Catalogue No. S7150)

The OxyBlot™ Protein Oxidation Detection Kit provides the chemical and immunological reagents necessary to perform the immunoblot detection of carbonyl groups introduced into proteins by oxidative reactions with ozone or oxides of nitrogen or by metal catalyzed oxidation.

Under the conditions recommended in the kit, as little as 5 femtomoles of carbonyl residue can be detected. This sensitivity is at least 100 times greater than that obtained by other procedures (such as radioisotope methodology utilizing [3H]-labeled $NaBH_4$). In addition, you can quantitatively analyze the oxidative status of each protein by comparing the signal intensity of the same protein in different lanes on the same or different gels.



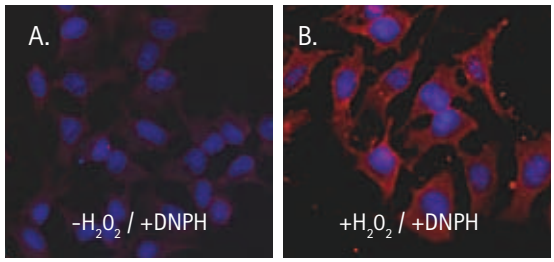
DNP in BSA Band (femtomoles)
Lane 1: 100
Lane 2: 30
Lane 3: 10
Lane 4: 3
Lane 5: 1
Lane 6: 0.3
Lane 7: 0.1

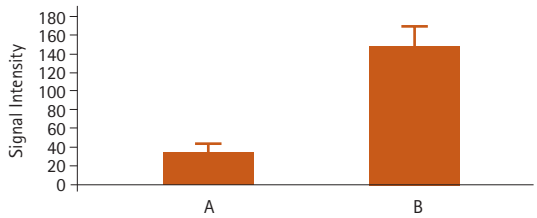
OxyICC™ Oxidized Protein Detection Kit

(Catalogue No. S7350)

Carbonyl formation is an important biomarker for oxidative stress. The OxyICC™ kit provides reagents for fluorescent immunocytochemistry of cellular protein carbonyls. This simple assay detects carbonyl modifications using dinitrophenylhydrazine (DNPH) to provide highly sensitive and quantitative results.

Quantitative immunodetection of protein carbonyls shows that peroxide treatment of cells results in over a four-fold signal intensity increase versus basal levels (B compared to A).





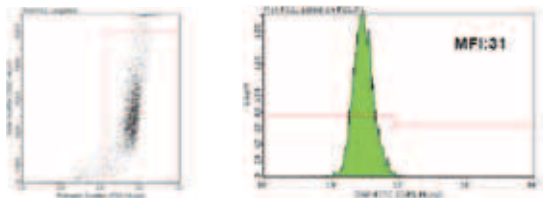
FlowCellect™ Oxidative Stress Characterization Kit

(Catalogue No. FCCH025111)

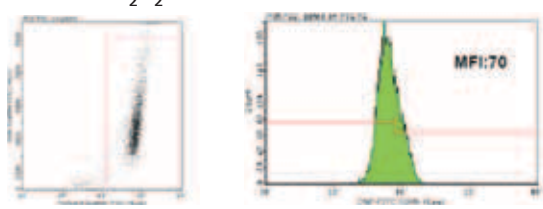
The new FlowCellect™ oxidative stress characterization kit enables flow cytometric quantification of oxidative stress within cells by providing all the reagents necessary to detect carbonyl groups introduced onto proteins by reactive oxygen species. Although the assay and all of the kit components are optimized for benchtop guava easyCyte™ instruments, you can use the kit with any flow cytometer equipped with a blue (488 nm) laser.

HeLa cells treated (B) with or (A) without hydrogen peroxide (H_2O_2) were processed using the FlowCellect™ oxidative stress characterization kit, then analyzed on a guava easyCyte™ flow cytometer.

Panel A: H_2O_2 Untreated Cells



Panel B: H_2O_2 Treated Cells



Key Products

Available from **www.millipore.com**

Antibodies

Description	Catalogue No.
Anti-Degraded Myelin Basic Protein (MBP)	AB5864
Anti-8-Hydroxydeoxyguanosine	AB5830
Anti-Nitrotyrosine	06-284
Anti-TRX	AB9328
Anti-SOD	AB5482
Anti-RAGE	AB5484
Anti-Neuroketal	AB5611
Anti-4HNE	AB5605
Anti-Malonidaldehyde ALDH	AB5524

Assays

Description	Catalogue No.
OxyICC™ Oxidized Protein Detection Kit	S7350
OxyELISA™ Oxidized Protein Quantitation Kit	S7250
Nitrotyrosine ELISA	17-10006
OxyIH Oxidized Protein Detection Kit	S7450

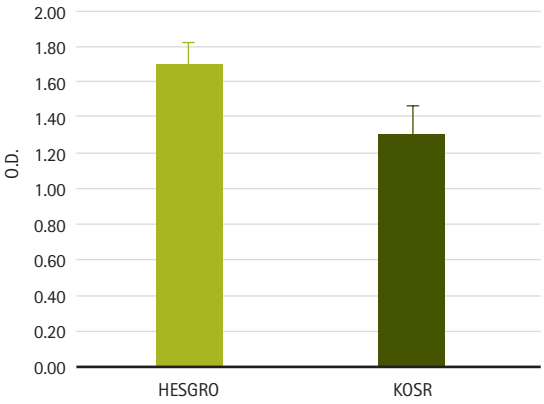
Mitochondrial Function Inhibitors

Description	Catalogue No.
Bongkrekic Acid, Triammonium Salt	203671
Carbonyl Cyanide m-Chlorophenylhydrazone	215911
Carboxyatractyloside, Potassium Salt, *Xanthium sibiricum*	216201
CGP-37157	220005
(-)-Deguelin, *Mundulea sericea*	252740
Erastin	329600
Hexokinase II Inhibitor II, 3-BP	376817
m-Iodobenzylguanidine, Hemisulfate	407721
Mitochondrial Division Inhibitor, mdivi-1	475856
Oligomycin	495455
Rotenone	557368
Ru360	557440

Proliferation and Viability

Evaluation of cell proliferation is essential for studies of most biological processes and for many cell-based assays. Measurement of [3H] thymidine incorporation as cells enter S phase has been a traditional method for detection of cell proliferation. Subsequent quantification of [3H] thymidine is performed by scintillation counting of autoradiography. This technology is slow, labor-intensive and has several limitations, including the handling and disposal of radioisotopes and the necessity of expensive equipment. EMD Millipore has developed multiple technologies for biomolecular detection and cellular analysis that offer significant advantages over [3H] thymidine incorporation for quantifying cell proliferation with speed, precision, and accuracy.

Featured Products



BrdU Cell Proliferation Kit

(Catalogue No. 2750)

The simple, nonradioactive kit is a well-established alternative to [3H] thymidine uptake for measuring proliferation. Bromodeoxyuridine (BrdU), a photoactivatable thymidine analog, is incorporated into newly synthesized DNA strands of actively proliferating cells. After exposure to UV light, DNA strands break at sites adjacent to incorporated BrdU. These sites are then labeled with TdT and Br-dUTP, and BrdU is detected with anti-BrdU, HRP-conjugated secondary antibody, and colorimetric detection.

Graph (Left): The BrdU cell proliferation kit (Catalogue No. 2750) was used to measure proliferation of H9 human embryonic stem cells in HEScGRO and KOSR medium, after cells were enzymatically expanded for 12 passages. Increased BrdU incorporation indicated faster cell proliferation in HEScGRO medium.

	Debris	Viable Cells	Apoptotic	Dead Cells
Forward Scatter	Low	High	High	High
Nuclear Stain	Neg	High	High	High
Viability Stain	Neg	Neg	Med	High

Guava ViaCount® uses two DNA binding dyes to identify viable, dead, and apoptotic cells.

Guava ViaCount® Reagent

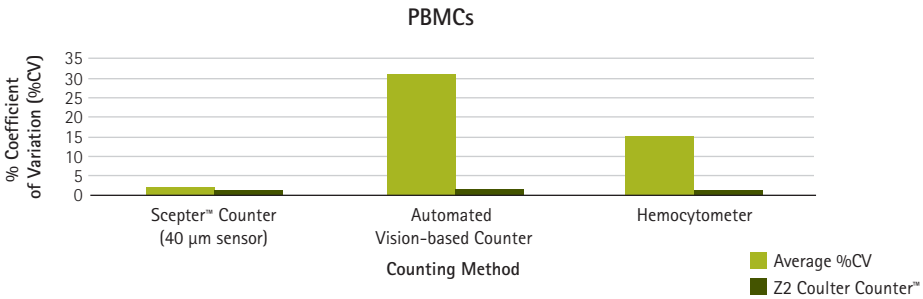
(Catalogue No. 4000-0040)

The ViaCount® assay provides rapid and reliable determinations of viability and total cell count using the power of flow cytometry. Precise, accurate assessments can be made with a wide variety of cell lines, even those with unusual culture conditions or a tendency to aggregate. A simple no-wash, mix-and-read procedure, the ViaCount® assay accurately determines absolute total cell counts, viability assessments, and apoptotic percentages with as little as 20 µL of sample.

Scepter™ Handheld Automated Cell Counter

(Catalogue Nos. PHCC20040 and PHCC20060)

Count cells and monitor proliferation with the first and only device to allow you to track your cell populations right at the culture hood. While other automated counters consume bench space and rely on object recognition software, manual focusing, and clumsy loading chambers, the Scepter™ cell counter provides true automation without the error that accompanies vision-based systems. With its microfabricated, precision-engineered sensor, the Scepter™ cell counter does all the work and delivers accurate and reliable cell counts in less than 30 seconds. Portable and precise, Scepter™ cell counter detects and measures the size of your cells, and displays the population as a histogram of cell size distributions. From the histogram, count all the cells or use the easy gating function to count a chosen subpopulation. Monitor histograms over time or after treatments for a quick and easy assessment of your cell population's health and proliferation rate.



The Scepter™ 2.0 cell counter counts PBMCs with greater precision than other counting methods, as reflected by low coefficients of variation. %CVs were calculated using average cell counts of four replicate samples.

TRAPeze® XL Telomerase Detection Kit

(Catalogue No. S7707)

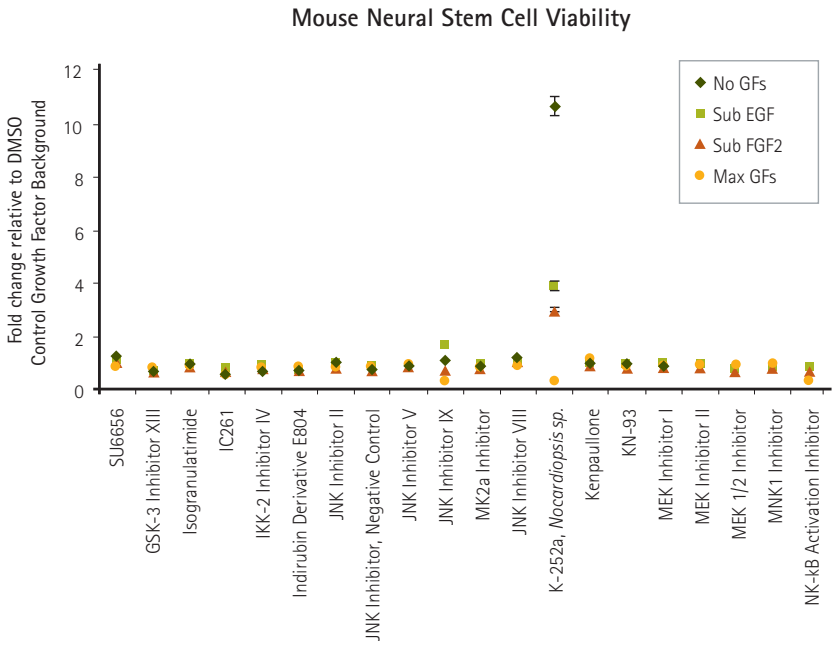
The telomerase enzyme maintains telomeres, DNA sequences at the ends of chromosomes that maintain cell viability and protect cells from the effects of aging. EMD Millipore provides a broad range of products for assaying telomerase activity. TRAPeze® Telomerase Detection kits are rapid, quantitative, in vitro assays for detecting activity. The TRAPeze® XL kit incorporates fluorescent primers.

Image demonstrates the direct fluorescence imaging of the TRAPeze® XL reaction of three specimens – telomerase positive lanes 1 and 2, and telomerase negative lane 3.

InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I

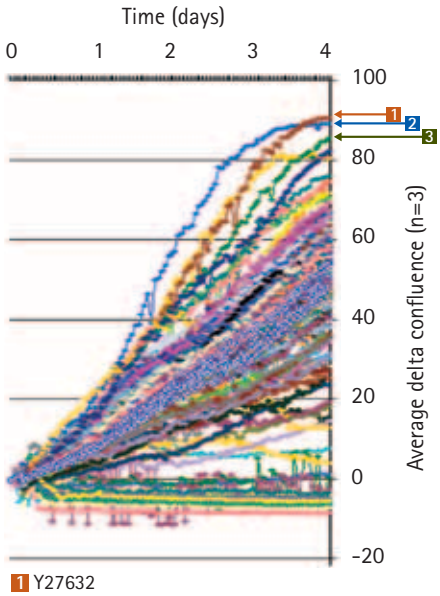
(Catalogue No. 539744)

This panel of compounds consists of 80, well-characterized protein kinase inhibitors targeting mainly tyrosine, AGC, and atypical families of kinases, the majority of which are cell-permeable and ATP-competitive. The library is useful for cancer signaling pathway analysis, cell-based assays, target identification in drug discovery, screening new protein kinases, and other related applications. It is supplied with a CD containing comprehensive documentation for each inhibitor.



InhibitorSelect™ 96-Well Protein Kinase Inhibitor Libraries I & II (160 inhibitors; Cat. Nos. 539744 and 539745) were screened for influence on proliferation and survival of mouse neural stem cells (mNS) in a cell viability assay under 4 conditions: (A) No GFs – No Growth Factors (to identify survival/proliferation factors) (B) Sub EGF – Sub-optimal EGF (to identify inhibitors/potentiators) 20 pg/mL EGF (C) Sub FGF2 – Sub-optimal FGF2 (to identify inhibitors/potentiators) 500 pg/mL FGF2 (D) Max GFs – Maximal EGF + FGF2 (to identify inhibitors/potentiators) 20 ng/mL EGF + 20 ng/mL FGF2 The presence of inhibitor K-252a, *Nocardiopsis sp.* (Cat. No. 420297) alone in the culture medium resulted in a 10-fold mNS cell viability.

Data courtesy of Donna McLaren, Stem Cell Sciences, Cambridge, UK



1 Y27632
2 Rho-kinase Inhibitor IV
3 HA 1077 dihydrochloride

InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library II

(Catalogue No. 539745)

This panel of compounds consists of 80, well-characterized, cell permeable, potent and reversible protein kinase inhibitors targeting mainly CMGC and CaMK families of kinases; the majority of which are ATP-competitive. This library is useful for target identification in drug discovery, biochemical pathway analysis, screening new protein kinases, and other pharmaceutical-related applications. It is supplied with a CD containing comprehensive documentation for each inhibitor.

Graph (left): Screening of 160 kinase inhibitors included in InhibitorSelect™ libraries I and II. Data show Delta Confluence Values, corresponding to the change in relative cell number for twelve mock-treated wells and 160 kinase inhibitors. Three compounds, all affecting Rho kinases, were selected as primary hits for their effect on expansion of NES cells and are detailed in the top right.

Key Products

Available from **www.millipore.com**

Antibodies

Description	Catalogue No.
Anti-TRF1, clone BED5 57-6	04-638
Anti-TRF2, clone 4A794	05-521

Assays

Description	Catalogue No.
Cell Proliferation Assay Kit, WST dye; ELISA based	2210
Cyclin B1 and Ki-67 Assay	HCS210
BrdU and Phospho-Histone H3 (Ser10) Assay	HCS212
guava® Nexin Assay	4500-0450
Brdu and Ki-67 Assay	HCS213
TRAPeze® Telomerase Detection Kit	S7700
TRAPeze® ELISA Telomerase Detection Kit, strip well plates	S7750
TRAPeze® RT Telomerase Detection Kit	S7710
TRAPeze® Telomerase Positive Control Cell Pellet	S7701

Inhibitors

Description	Catalogue No.
Actinomycin D, 7-Amino	129935
Aphidicolin	178273
HSV Replication Inhibitor, BP5	385883
Novobiocin, Sodium Salt	491207
RNA Polymerase III Inhibitor	557403

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The Muse™ cell analyzer delivers accurate cell counts and quantitative assessments of cell viability, apoptosis and cell cycle in just minutes, enabling you to make faster, better decisions about your experiments, accelerating your research.

Muse™ Advantages:

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- Ultra-compact size: Patent-pending design for miniaturized fluorescent optics.
- Validated assays and revolutionary touchscreen interface: Rapid setup, effortless operation, intuitive software.

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