MERCK MILLIPORE

Advancing cancer research:
From hallmarks & biomarkers to tumor microenvironment progression

Merck Millipore is a division of MERCK

Platforms, Technologies, and Services

As a tools provider and partner in research, Merck 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. Chemical genetics, in which loss of function is imposed using small molecules, can reveal mechanisms of tumorigenesis and tumor progression. Merck 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.

ANTIBODIES AND IMMUNOASSAYS

With the expertise of Upstate® and Chemicon®, Merck 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. Merck Millipore also offers a variety of ELISAs in major research areas, including cancer research, and novel tools for improving the Western blotting workflow.

CELL-BASED ASSAYS

Our portfolio of live cell, whole-cell and cell-based activity assays and reporter systems advances direct and indirect detection of signaling and other cellular processes. These technologies facilitate protein target validation, identify cellular pathways and determine mechanism of action for lead optimization environments.

FLOW CYTOMETRY ASSAYS AND SYSTEMS

Simultaneously measuring multiple parameters on individual cells, flow cytometry is essential for in-depth cell analysis. Our Amnis® imaging flow cytometers combine the speed, sensitivity, and phenotyping abilities of flow cytometry with the imagery and functional insights of microscopy, taking cancer research to higher levels of discrimination and discovery. Our easyCyte™ flow cytometers provide precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. Validated FlowCellect™ assay kits, Milli-Mark™ conjugated antibodies and application-specific software modules provide a complete solution for flow cytometry.

MILLIPLEX® MAP MULTIPLEX ASSAYS

Isolated biomarkers are often inadequate to distinguish tumor from non-tumor. Multiplexed detection of both intracellular and circulating cancer biomarkers can accelerate studies of normal homeostasis and tumorigenic processes. Using the Luminex® xMAP® platform, MILLIPLEX® MAP multiplexed immunoassay panels meet your research needs with a broad offering of soluble cancer biomarker assays, inflammation and immune response cytokine/chemokine panels, cellular metabolism, traditional cell signaling kits and MAPmate™ cell signaling assays. Our MILLIPLEX® MAP portfolio is backed by a complete spectrum of Luminex® instrumentation, software, service and support.

PROTEIN PURIFICATION AND PREPARATION

For every step of the molecular biology and protein workflow, from cloning DNA targets to purifying and concentrating recombinant proteins, Merck Millipore provides reagents, kits, cells and tools that are specifically designed to meet your scientific and technical goals. For protein quantitation, the infrared-based Direct Detect™ spectrometer distinguishes proteins and peptides from interfering sample components, providing more accurate results without the pitfalls of colorimetric assays.

CELLS AND CELL CULTURE

Merck Millipore's innovative cell culture solutions help optimize cell growth and maintenance for cancer research. 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. Our flexible sterile filtration devices offer fast flow and have many membrane options. Also available are microfluidic systems to mimic in vivo conditions, provide coculture options and facilitate live cell imaging.

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Introduction

Comprehensive Solutions from Hallmarks to Biomarkers

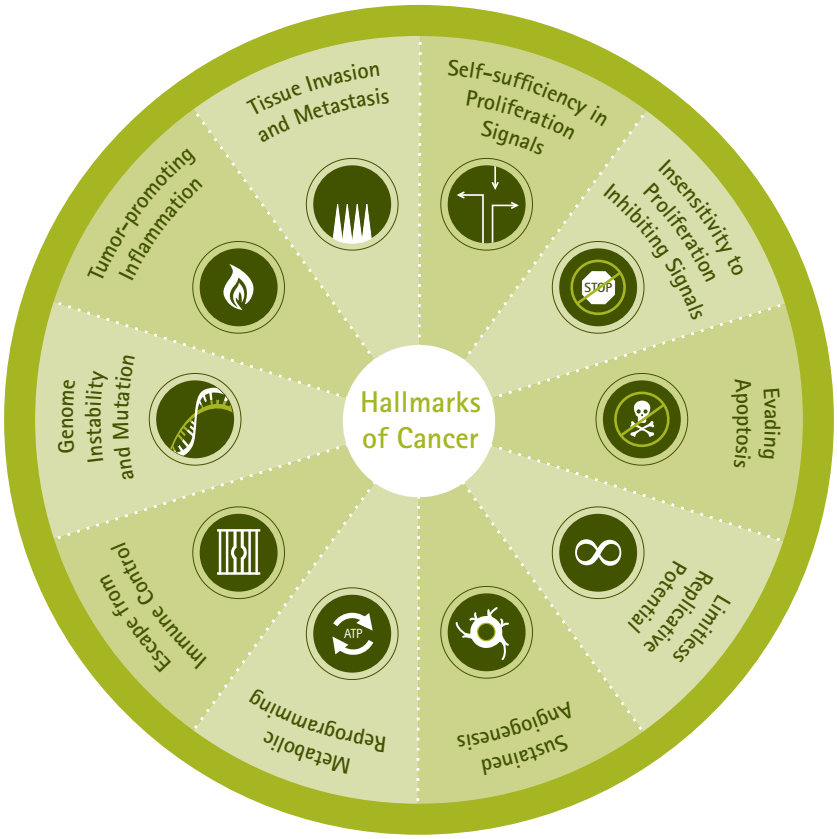
Our understanding of cancer is rooted in identifying the phenotypic differences between cancer cells and corresponding normal cells of the same lineage. Although the recent, extensive output of comparative genomic, proteomic, and epigenomic data comparing tumor and non-tumor cells has yet to be fully mined, the data points to ten key traits, shared by most tumor types, that drive disease progression. These are listed in the table of contents (left).

These "hallmarks" of cancer, as described by Hanahan and Weinberg[1], are important—not only because they represent opportunities for therapeutic intervention, but also because they are opportunities to use tumors as models to decipher the signaling pathways underlying both normal and diseased cellular processes. As a result, cancer research not only impacts drug discovery, but also advances understanding of all of biology, from developmental mechanisms to immune response and aging.

Although it is tempting to view these ten hallmarks as a linear path ending in metastatic cancer, challenges such as tumor heterogeneity, individual polymorphisms, nonparallelism in model systems, and inability to distinguish cell-intrinsic from cell-extrinsic factors require an unbiased, multidisciplinary, multiparametric, cross-platform approach to cancer research.

Recognizing both the tremendous opportunities and the challenges facing cancer research, Merck Millipore has been dedicated to developing and refining tools and technologies for the study of cancer. With Merck Millipore's comprehensive portfolio, including the Upstate®, Chemicon®, and Calbiochem® brands of reagents and antibodies, researchers can count on dependable, high quality solutions for analyzing all the hallmarks of cancer.

Reference
1. Hanahan D., Weinberg R.A. Hallmarks of Cancer: The Next Generation. Cell. 144(5):646-74.



Featured Products

- Ras, Rac1, Rho, Rac1/Cdc42 Activation Pulldown Assay Kits
- Calbiochem InhibitorSelect™ Protein Kinase Inhibitor Libraries
- ApopTag® Family of TUNEL Assays & CaspaTag® Family of Active Caspase Detection Products for Apoptosis
- Calbiochem® BrdU Cell Proliferation Assay
- *In Vitro* Vascular Permeability Assays (24- & 96-Well)
- LentiBrite™ GFP- & RFP-LC3 Lentiviral Biosensors
- LC3-II Enrichment Kits (Western Blot & Flow Cytometry)
- MILLIPLEX® MAP Human Circulating Cancer Biomarker Panel 1
- TRAPeze® Telomerase Detection Kits

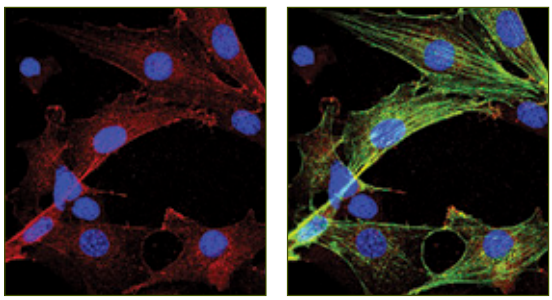
Self-Sufficiency in Proliferation Signals

Normal cell proliferation is commonly regulated by cell signaling pathways that lead to reversible modification of existing proteins and phospholipids. These modifications, which include phosphorylation, methylation, acetylation, ubiquitination, and hydroxylation, function in combination to control individual proteins or multi-protein pathways. While normal cells require extracellular cues for cell division, cancer cells possess mutations, often due to genetic instability, that render their proliferation independent of these cues. Rapid proliferation, in turn, provides more opportunities for tumor cells to acquire mutations favoring tumor progression.

Anti-mTOR, clone 2ID8.2

(Catalogue No. 05-1592)

mTOR affects cell proliferation and survival primarily by phosphorylating Akt, an effector of the PI3 kinase pathway. In addition, mTOR regulates cell growth and autophagy. The mTOR pathway is frequently dysregulated in cancer. Validated for Western blotting and immunocytochemistry, anti-mTOR is one of our numerous antibodies for studying self-sustained proliferation, including key receptors such as EGFR, ErbB2, Met, VEGFR, and IR and downstream targets such as Ras, PI3-Kinase, Akt, Mek, and Erk.

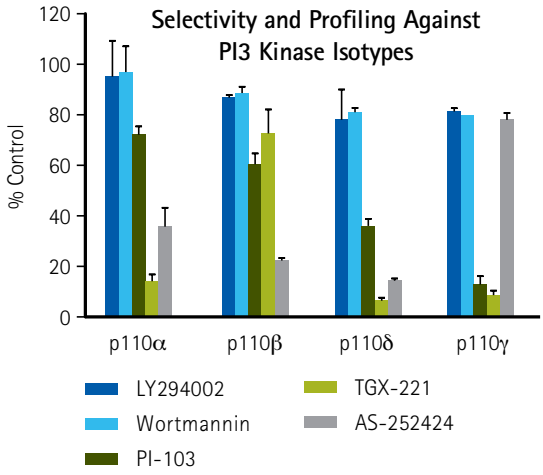


Confocal immunofluorescence analysis of NIH 3T3 cells using anti-mTOR (05-1592) (red) shows diffuse cytoplasmic distribution. Actin filaments have been labeled with Alexa Fluor® 488-Phalloidin (green). Nuclei are stained with DAPI (blue).

PI3 Kinase Activity/Inhibitor ELISA

(Catalogue No. 17-493)

The discovery of tumor-specific mutations in PI3 kinase (PI3K) genes has shifted research focus on PI3K from basic biochemistry to target validation and drug development. These mutations single out class I PI3K as an important contributor to oncogenesis. Most human cancers also show activated PI3K signaling. Easily evaluate PI3K activation and inhibition with this competitive ELISA kit. The PI3 Kinase Activity/Inhibitor Assay enables fast and sensitive quantitation of activity of the four class I PI3 kinases (p110 α, β, δ, γ).



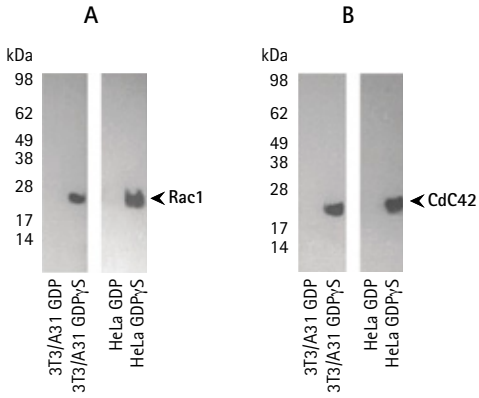
Results from the PI3K ELISA kit demonstrate inhibition via isoform-specific and general class I inhibitors.

Ras, Rac1, Rho, Rac1/Cdc42 Activation Pulldown Assay Kits

(Catalogue Nos. 17-218, 17-283, 17-294, 17-441)

Small GTPases are key conductors of signals emanating from the cellular membrane to intracellular compartments. They stimulate intracellular kinase cascades, cytoskeleton reorganization, and vesicular/organellar translocation. Mutations in Ras & overexpression of Rho, Rac, and Cdc42 has been shown in multiple types of cancer. Using Raf1 RBD, PAK1 PBD, and Rhotekin RBD agarose beads as well as corresponding antibodies, fast and effective pulldown of Ras-, Rac1-, Rho-, and Rac1/Cdc42-GTP can be achieved.

Magnetic bead versions also available.

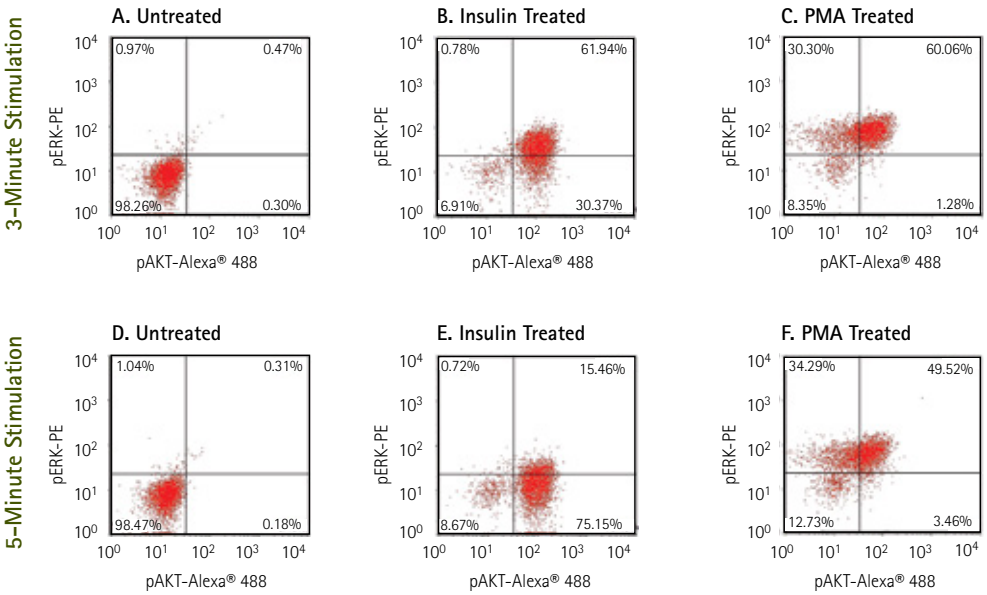


Efficient pulldown/immunoprecipitation of only GTP-Rac1 & GTP-Cdc42 was achieved from 3T3/A31 and HeLa cell lysates incubated with either GDP or GTPγS (non-cleavable GTP).

FlowCellect™ PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection Kit

(Catalogue No. FCCS025100)

The two kinases PI3K and MAPK often mediate aberrant cell proliferation, and recent research suggests cross-talk among their downstream effectors. This flow cytometry kit enables easy analysis of the role of cross-talk in proliferation by providing three fully validated and optimized antibodies to measure specific cell signaling events along both pathways. The kit uses directly labeled antibodies against phospho-Akt and phospho-ERK1/2 to analyze signaling activation and cross-talk, plus a Ki-67 marker to identify the proliferative fraction.

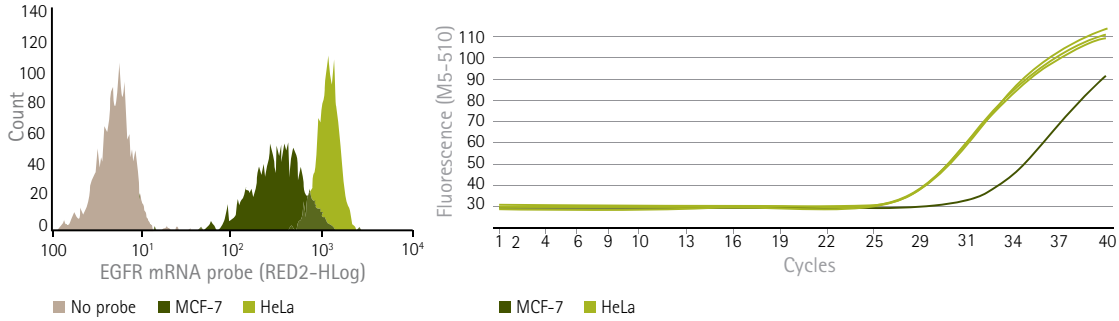


HEK293 cells were stimulated by insulin (B, E) or PMA (C, F) and simultaneously stained with antibodies against phospho-Akt and phospho-ERK1/2. The cross-talk between the PI3K and MAPK signaling pathways is demonstrated by the sharp decrease in ERK phosphorylation after five minutes of insulin stimulation.

SmartFlare™ EGFR Hu-Cy5 RNA Detection Reagent

(Catalogue No. SF-151)

Epidermal growth factor receptors (EGFR) play crucial roles in regulating cell proliferation, differentiation, motility, and apoptosis, contributing to cancer and other pathological processes. Mutations in EGFR have been correlated with many cancers. SmartFlare™ reagents enable measurement of EGFR expression inside individual, live cells, using flow cytometry, microscopy or other cell analysis techniques. As a result, once-difficult studies of tumor heterogeneity caused by cell-to-cell variations in gene expression can now be easily explored. Learn more about new SmartFlare™ technology at: **www.millipore.com/smartflare**

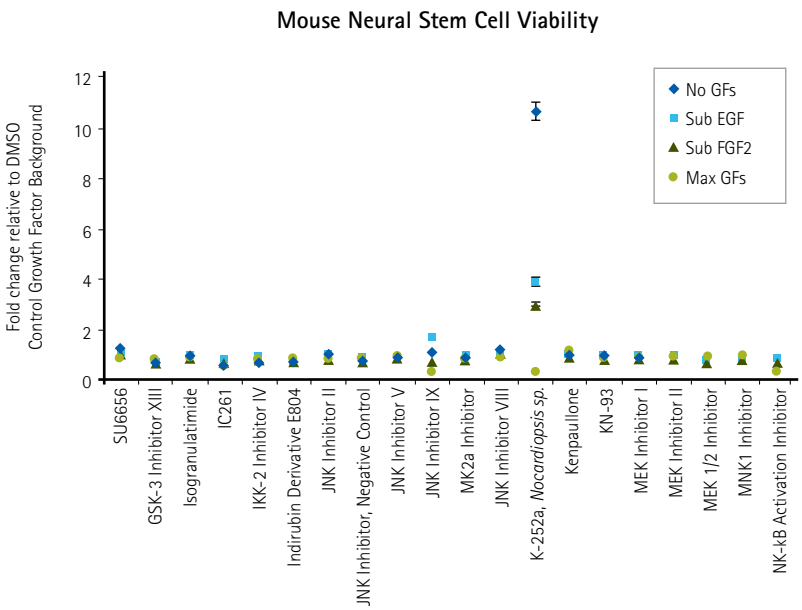


SmartFlare™ probe detection of mRNA levels correlates to qRT-PCR data. Using SmartFlare™ technology to determine the mRNA levels of EGFR mRNA in HeLa and MCF-7 cells correlate with EGFR mRNA levels measured by qRT-PCR. Flow cytometry provides added information at the single cell level as well as how the expression is distributed within the population.

Calbiochem InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I

(Catalogue No. 539744)

This panel of compounds consists of 80, well-characterized protein kinase inhibitors, 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; Catalogue 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. (Catalogue 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

Lead Discovery Profiling Services

Mutations of many receptor tyrosine kinases, G protein-coupled receptors, and intracellular kinases are responsible for uncoupling the requirement for external proliferation cues in cancer cells. Many small molecule GPCR and kinase inhibitors have been successfully been developed as anti-tumorigenic drugs. Merck Millipore's Lead Discovery Services are well suited to extensive profiling of these drugs against a broad range of targets to reveal interactions that may have additional therapeutic value or present adverse reactions.

GPCRProfiler® Service

GPCRProfiler® is the first complete cell-based functional platform that uses a common validated readout for over 155 GPCRs. The foundation of GPCRProfiler® is Merck Millipore's ChemiScreen® GPCR stable cell lines that are used for real-time calcium flux assays to rapidly, reliably and reproducibly screen and profile compounds. Using one platform allows ligands to be screened with identical buffer conditions and incubation times for the entire spectrum of GPCRs for easy analysis and comparison.

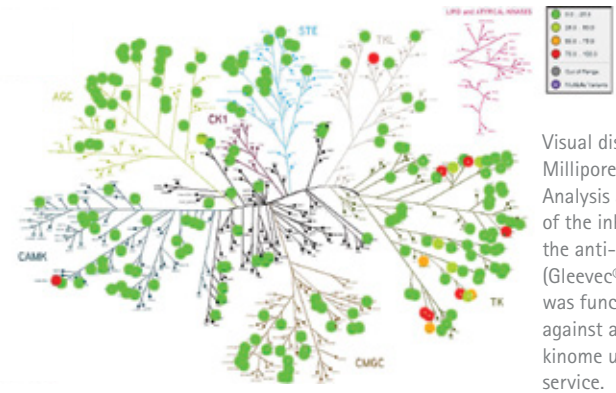
Quickly implement screening in a new disease area or target receptor, or increase your current screening capacity with GPCRProfiler® services. Our unbiased profiling screens can determine if drugs are full or partial agonists or antagonists. We also perform dose-response assays to determine EC_{50} values for agonists and IC_{50} values for antagonists. You will typically receive results within 1-3 weeks of compound submission, in a thorough and intuitive report. GPCRProfiler® gives you the flexibility to choose from 1 to ≥155 different receptors to screen. Or, choose between 15 different service panels, including more than 60 distinct ligand families, 2 safety panels and 11 disease-focused panels.

Kinase & Phosphatase Profiling Services

In 2000, the KinaseProfiler™ service developed by Upstate, now a part of Merck Millipore, brought selectivity profiling to kinase drug discovery researchers. Today, the KinaseProfiler™ panel includes almost 300 protein and lipid kinases, 21 phosphatases and a complementary suite of secondary assays, forming the most diverse, disease-relevant panel available commercially. As the partner of choice for kinase screening, we provide validated data using the robust and reliable radiometric kinase assay trusted by the world's leading pharmaceutical companies.

SignalProfiler™ Services

Providing fresh insights to the cellular activity of compounds, SignalProfiler™ service employs MILLIPLEX® MAPmate™ cell signaling kits to profile samples against a panel of 90+ modified and total proteins. SignalProfiler™ services can be used to measure the effectiveness of kinase inhibitors and GPCR ligands by measuring changes in signaling cascades in relevant cell backgrounds. In addition, early signaling events leading to cellular toxicity can be studied to help assess potential compound liabilities in early stages of drug development.



Visual display using Merck Millipore's DART™ (Data Analysis and Reporting Tool) of the inhibitory activity of the anti-cancer agent imatinib (Gleevec®, Novartis) which was functionally profiled against a large portion of the kinome using KinaseProfiler™ service.

UbiquitinProfiler™ Service

Recognizing that dysregulation of the ubiquitin/proteasome pathway in cancer, Merck Millipore launched UbiquitinProfiler™, the first service to provide compound screening and profiling against E3 ligase cascades. Each ubiquitination cascade on the UbiquitinProfiler™ panel consists of the three enzyme components (E1 activating enzyme, E2 conjugating enzyme and E3 ligase), plus a biologically-relevant substrate. The incorporation of ubiquitin into the substrate is quantitatively detected by electrochemiluminescence, which allows for the identification of compound inhibitors and activators.

Key Products for Proliferation Signaling

Antibodies and Proteins

Description	Catalogue No.
Anti-phospho-erbB-2/HER-2 (Tyr1248)	06-229
Anti-Ras, (K-, H-, N-), clone 9A11.2	05-1072
Anti-Ras-related protein Ral-A	07-2132
EGFR, active, purified kinase	14-531

For our entire line of cell signaling antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
PIP3 Quantification TR-FRET Assay	17-494
c-ErbB2/c-Neu Rapid Format ELISA Kit	QIA10
Phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) STAR ELISA Assay Kit	17-464
Raf-1 Kinase Assay Kit, Chemiluminescence Detection	17-360
MILLIPLEX® MAP Human Mitogenesis RTK 8-Plex, Phosphoprotein	48-672MAG
MILLIPLEX® MAP Human Mitogenesis RTK 8-Plex, Total Protein	48-671MAG
MILLIPLEX® MAP Human EGFR Profiler 8-Plex	48-613MAG
FlowCellect™ MAPK Activation Dual Detection kit	FCCS025106
FlowCellect™ PI3K Activation Dual Detection Kit	FCCS025105
FlowCellect™ EGFR/MAPK Pathway Activation Detection Kit	FCCS025101
K-LISA™ mTOR (Recombinant) Activity Kit	CBA104
PI3-Kinase HTRF Screening Assay	33-016

Compound Libraries

Description	Catalogue No.
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library II	539745
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library III	539746
InhibitorSelect™ 384-Well Protein Kinase Inhibitor Library I	539743
InhibitorSelect™ 96-Well Tyrosine Kinase and Phosphatase Inhibitor Library IV	539747
StemSelect® Small Molecule Regulators 384-Well Library I	569744

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

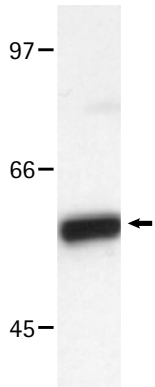
Insensitivity to Proliferation Inhibiting Signals

To maintain normal cellular homeostasis, proliferation inhibiting signals, including soluble inhibitors and inhibitors residing on the surface of nearby cells, must be received and transmitted through intracellular pathways. Adaptation through mutation may render tumor cells resistant to these proliferation inhibiting signals, enabling them to overcome cell cycle checkpoints. These aberrant signal transduction networks can either induce or support tumor cell development and proliferation in an organism. Cell cycle signaling through retinoblastoma (Rb) and TGFb are two widely studied pathways upon which proliferation inducing signals converge.

Anti-Cyclin B1, clone GNS3 (8A5D12)

(Catalogue No. 05-373)

Cyclin B1 triggers mitosis by binding and activating cdc2 kinase, which phosphorylates multiple protein targets. Validated for immunoprecipitation and Western blotting, this monoclonal antibody complements Merck Millipore's huge portfolio of transcription factor antibodies, including other important cell cycle regulators, including Rb, Wee1, and CDCs. Merck Millipore also offers numerous Antibody Minipacks supporting various cell cycle stages and DNA repair pathways that enable you to explore more for less.



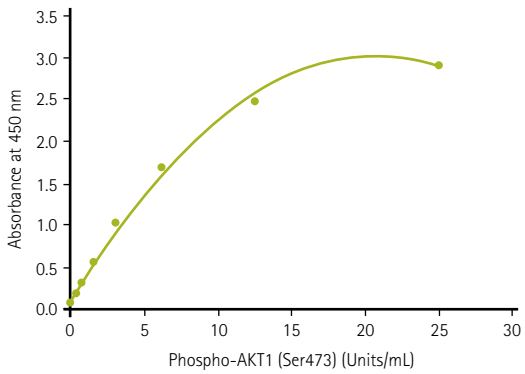
Non-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Cyclin B1 (05-373, 0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Cyclin B1 (~58 kDa).

Phopsho-Akt (Thr473) STAR ELISA Kit

(Catalogue No. 17-457)

Akt (Protein Kinase B), a Ser/Thr kinase, is a key effector of the PI3 Kinase pathway and is involved in multiple signaling pathways that relate to glucose metabolism, cell survival/apoptosis, cell cycle control, angiogenesis, differentiation, cell growth and proliferation and other processes.

The colorimetric STAR (Signal Transduction Assay Reaction) ELISA kit is a solid phase sandwich enzyme-linked immunosorbent assay that provides a fast, sensitive method to detect specific levels of signaling targets in whole cell extracts.



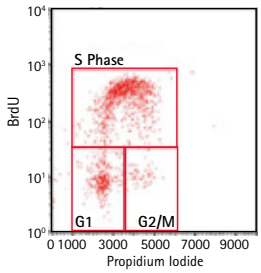
NIH3T3 cells were seeded at $1x10^4$ cells per well, cultured for 24 hr, serum starved 16 hr, and stimulated with 50 ng/mL of PDGF for 0, 1, 5, 15, or 30 minutes. Total and phospho Akt levels were determined using Akt (Ser473) Dual Detect CELISA Kit (17-444). Phosphorylated Akt levels are normalized to the total Akt. Inset shows the corresponding Western blot.

FlowCellect™ Bivariate Cell Cycle Kit for DNA Replication

(Catalogue No. FCCH025102)

Flow cytometry is an accurate, high-throughput, high content method for studying proliferating cells undergoing DNA replication in the S-phase of the cell cycle. The kit includes a directly conjugated Anti-BrdU Alexa Fluor® 488 conjugate plus a DNA dye (propidium iodide) which enable bivariate analysis of proliferating cells without the need of software modules for data interpretation.

Detection of DNA replication by analysis of S phase cells. Bivariate flow cytometric analysis using BrdU Alexa Fluor 488 conjugate can distinguish S phase cells with great accuracy, not only based on their difference in DNA content from G1 or G2/M cells but also as having incorporated BrdU.



BrdU Cell Proliferation Kit (200 & 1000 Assays)

(Catalogue Nos. 2750 and 2752)

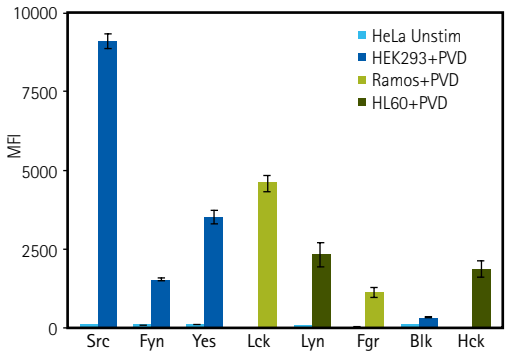
Incorporation of BrdU into cellular DNA correlates with cell proliferation rate and the number of cells in S phase. This BrdU Cell Proliferation Assay Kit is a non-isotopic assay for the *in vitro* quantitative detection of newly synthesized DNA of actively proliferating cells.

MILLIPLEX® MAP Human Src Family Kinase Active Site Magnetic Bead Multiplex Assay – 8-Plex

(Catalogue No. 48-650MAG)

SFKs play crucial roles in embryonic development and cell proliferation, regulating such functions as adhesion, differentiation, and survival. These proteins are often involved in the progression and oncogenesis of non-small cell lung cancer (NSCLC), squamous cell head and neck and pancreatic cancers. Merck Millipore's MILLIPLEX® MAP Src Family Kinase 8-Plex is the only multiplex cell signaling kit available able to assess and distinguish the phosphorylation status of each SFK.

A.



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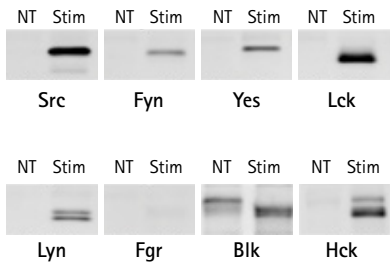


Phospho-SFK detection in cell lysates. Tumor cells (Ramos, HL-60 and HEK293) were treated with sodium pervanadate to enrich for phosphorylated proteins. Cell lysates prepared from untreated and pervanadate-treated cells were incubated with a mixture of all eight antibody-coated magnetic beads. Tyrosine phosphorylation of SFKs was detected with antiphosphotyrosine antibodies. Multiplex analysis (A) showed Lck and Fgr phosphorylation in Ramos cells; Src, Fyn, Yes and Blk phosphorylation in HEK293 cells; and Lyn and Hck phosphorylation in HL60 cells, respectively. The confirmatory immunoprecipitation/Western blots are shown in (B).

Calbiochem InhibitorSelect™ Protein Kinase Inhibitor Libraries

Interrogate multiple proliferation and survival signaling pathways simultaneously and efficiently by using InhibiorSelect Protein Kinase Inhibitor Libraries. Targets (see comprehensive table below) include kinases important for cancer research, such as Aurora, Bcr-Abl, Ras, Rho, and Wee1.

Inhibitor Characteristics

- Cell-permeable*
- Potent and selective*
- ATP-competitive*
- Reversible*
- Stable in DMSO
- Structurally diverse
- Some target multiple kinases
- Known pharmacological activity
- Less toxic

Highest Quality Control

- Purity by HPLC (≥95%)*
- Lot-specific data for every inhibitor in solution
- DMSO-resistant polypropylene deep-well microplates

CD-ROM with comprehensive data set included in each library

- Inhibitor description
- Published IC_{50}/K_i values
- Literature citations
- SD files
- CAS numbers
- PubChem compound ID
- Molecular weight
- Molecular structure

*Pertains to the majority of inhibitors.

Target Kinases	96-well Library I (Cat. No. 539744)	96-well Library II (Cat. No. 539745)	96-well Library III (Cat. No. 539746)	96-well Library IV (Cat. No. 539747)	384-well Library I (Cat. No. 539743)
ADK	x		x		
Akt	x		x		x
AMPK		x			x
ATM		x			x
ATR		x			x
Aurora	x	x	x		x
Bcr-Abl	x			x	x
B-Raf				x	
BTK				x	
CaMK		x	x		x
CD45				x	
Cdks		x	x	x	x
cFMS	x				x
cFMSR				x	
Chk1,2		x	x		x
CK1,2		x	x		x
c-Met	x			x	x
c-Met/Ron				x	
C-Raf				x	
C-Src				x	
DAG	x				x
DNA-PK	x		x		x
eEF2	x		x		
EGFR	x			x	x
EGFR/ErbB2/ErbB4				x	
EGFR/FGFR/PDGFR				x	
EGFR/PDGFR/IGF-1R				x	
ErbB2				x	
ERK		x	x		x

Target Kinases	96-well Library I (Cat. No. 539744)	96-well Library II (Cat. No. 539745)	96-well Library III (Cat. No. 539746)	96-well Library IV (Cat. No. 539747)	384-well Library I (Cat. No. 539743)
FGFR				x	
FGFR/PDGFR/VEGFR				x	
FGFR/VEGFR1				x	
FGFR3				x	
Flk-1				x	
FLT3	x			x	x
Flt3/TrkA				x	
GFRs				x	
GSK-3		x	x		x
IGFR	x				x
IKK		x	x		x
IP3K	x		x		
IRAK	x				x
JAK	x			x	x
JNK		x		x	x
LcK	x			x	x
Lck/Fyn/Hck/Src				x	
LYP				x	
MAPK		x	x		x
MEK		x	x		x
Met/Flt-3/VEGFR2				x	
MLCK		x	x		
MNK1	x				x
p70 S6	x				x
p90 S6	x		x		
PAK	x		x		
PDGFR	x			x	x
PDGFR/FGFR				x	
PDGFR/VEGFR2				x	
PDK1	x				x
PI 3-K	x		x		x
PIKfyve	x		x		x
PIM	x		x		
PKA	x		x		
PKC	x		x	x	x
PKG	x		x		
PKR	x				x
PLK	x		x		
Polo/ZAP-70/Syk mutants				x	
PRL-3				x	
pSTAT3				x	
pSTAT5				x	
PTK/PI 3-K/mTOR				x	
PTP1B				x	
PTP1B/SHP-1/PTPases				x	
Rafs				x	
RET RTK				x	
Rho	x		x		x
RIP		x	x		
SHP-1/SHP-2				x	
SHP-2				x	
SHP-2/PTP1B/PTPases				x	
SHP-2/PTP1B/TCPTP				x	
SK	x			x	x
Src	x	x			x
Syk	x	x			x
TGF-βR	x				x
Tie2				x	
TP-12	x		x		
TrkA				x	
VEGFR	x				x
VEGFR2				x	
Wee1	x		x		

Key Products for Proliferation Checkpoint

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
Akt Magnetic Bead conjugate	16-318
Anti-Cdc42-interacting protein 4	ABS69
TGFBR-1, active	14-912

For our entire line of cell proliferation signaling antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
MILLIPLEX® MAP Multi-species TGFb 3-Plex	TGFB-64K-03
MILLIPLEX® MAP TGFb (beta) Signaling Pathway 6-Plex	48-614MAG
MILLIPLEX® MAP MAPmate™, retinoblastoma Thr252	46-704
FlowCellect™ Bivariate Cell Cycle Kit for G2/M Analysis	FCCH025103
guava® Cell Cycle Reagent Propidium Iodide Solution	4500-0220
Muse™ Cell Cycle Kit	MCH100106
SmartFlare™ miR-155-5p Hu-Cy3	SF-183

Inhibitors

Description	Catalogue No.
Chk2 Inhibitor II	220486
SB 218078	559402
Staurosporine	569397
Cdk1 Inhibitor IV, RO-3306	217699

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

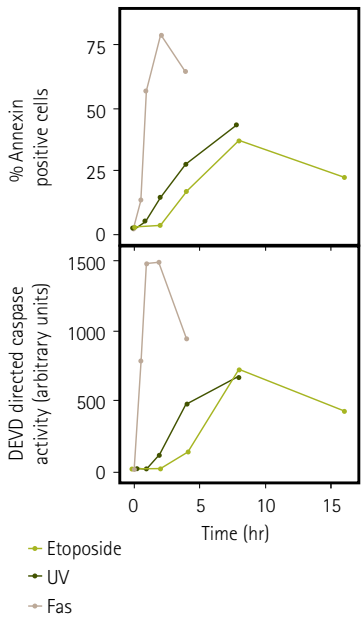
Evading Apoptosis

The ability to elude programmed cell death (apoptosis) is a hallmark of most types of cancer. A variety of proteins, including cell surface receptors, adaptors, proteases and mitochondrial components, regulate a fine balance between cell survival and death by apoptosis. Mutations of these proteins may tip the balance, resulting in the uncontrolled survival and proliferation of cancer cells. In many cancers, aberrant regulation results in overexpression of survivin, a potent anti-apoptotic protein. Recent studies also show that autophagy (see Technology Highlight), the lysosomal breakdown of a cell's own components, exhibits cross-talk with apoptotic pathways, and autophagy-deficient cells may be prone to tumorigenesis. Understanding apoptotic and autophagy signaling and mechanisms can help exploit these pathways for therapeutic benefit.

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.

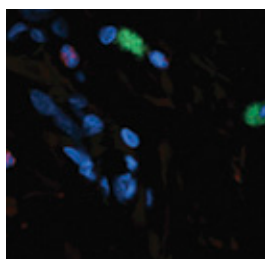


Etoposide-, UV-, and Fas-mediated apoptosis in Jurkat cells. Apoptotic response, as measured by annexin V binding (top) and caspase activity (bottom), was the fastest upon stimulation with anti-Fas antibody (05-201). This research was originally published in the Journal of Biological Chemistry. Widmann,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

ApopTag™ ISOL Dual Fluorescence Apoptosis Detection Kit

(Catalogue No. APT1000)

During apoptosis, one class of DNases, which includes DNase I, is activated via caspase signaling, while the other class, which includes DNase II, is activated via a caspase-independent pathway. The ApopTag™ ISOL Apoptosis Kit facilitates the differentiation of apoptotic cells from necrotic or transiently damaged cells by using a proprietary dual fluorescent-labeled oligonucleotide to detect simultaneously both DNase I and DNase II-type DNA fragments in a single sample, thus distinguishing caspase-dependent and caspase–independent apoptotic events. While conventional in situ detection techniques such as ISEL (Klenow DNA polymerase), TUNEL (terminal deoxynucleotidyl transferase, TdT) and ISNT (DNA Polymerase I) are useful in detecting internucleosomal DNA cleavage, they do not differentiate DNase Type I and DNase Type II cleavage. The ISOL technique shows concordant results with the TUNEL technique in specimens without necrosis, and in specimens presenting necrosis, the ISOL technique shows improved selectivity as compared to TUNEL.

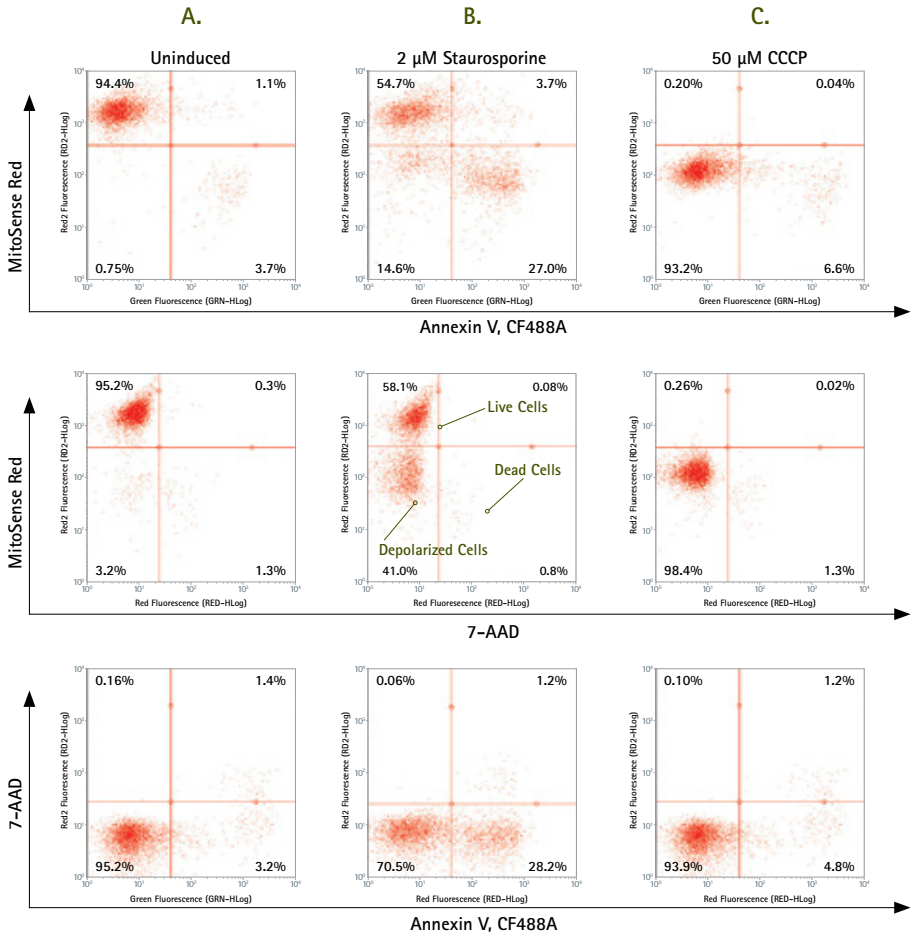


Detection of caspase-dependent (red) and caspase-independent (green) DNase activity in paraffin-embedded rat mammary gland tissue sections using ApopTag™ ISOL Dual Fluorescence Apoptosis Detection Kit (APT1000).

FlowCellect MitoDamage Kit

(Catalogue No. FCCH100106)

Multiparametric, flow cytometric evaluation of apoptosis markers enables detailed kinetic and temporal analysis of the events that lead to apoptosis, both in individual cells and cell populations. This kit enables the simultaneous measurement of 3 important cell health parameters; change in mitochondrial potential (an early hallmark of apoptosis and cellular stress), phospatidylserine expression on the cell surface of apoptotic cells as assessed by Annexin V binding and plasma membrane permeabilization (indicating cell death).



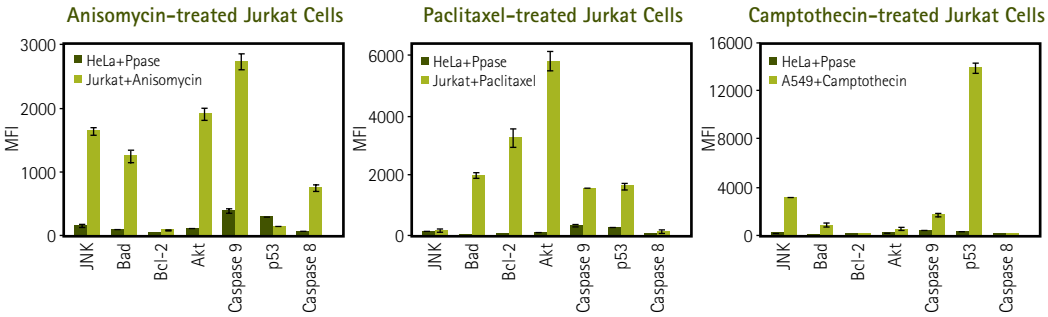
Jurkat cells were uninduced (A), induced to apoptosis with staurosporine (B) or with CCCP (C), then stained using the MitoDamage kit. Flow cytometry showed that staurosporine induced apoptosis in Jurkat cells, and that CCCP depolarized the mitochondrial membrane, but neither condition was sufficient for cell membrane permeabilization and death.

MILLIPLEX® MAP Human Early and Late Apoptosis Magnetic Bead 3- & 6-Plex Multiplex Assays

(Catalogue Nos. 48-669MAG and 48-670MAG)

Apoptosis depends on coordinated regulation by multiple, interconnected pathways, therefore, it requires understanding the roles of pathway proteins in both normal apoptosis and dysregulated cancer cells, which necessitates the quantitation of multiple biomarkers in each sample. A portolio of MILLIPLEX® MAP panels and MAPmate™ enables the accurate, Luminex® technology-based detection of expression of key apoptosis proteins.

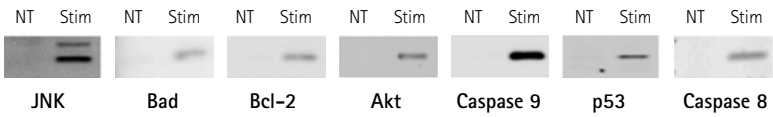
A.



(A). 7-Plex early apoptosis bead kit analysis. Multiplex analysis of Jurkat and A549 cells treated with anisomycin, paclitaxel, or camptothecin.

(B) Immunoprecipitation/ Western Blot analysis of multiplexed analytes in various cells.

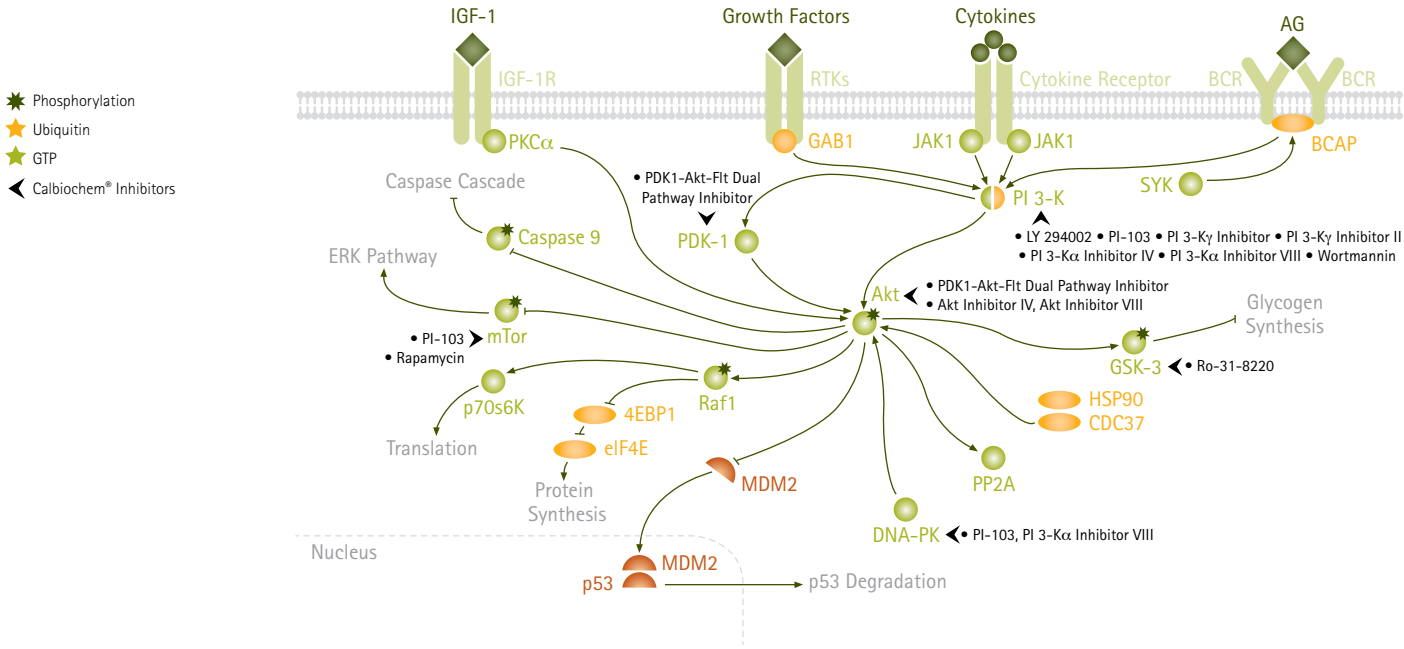
B.



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

(Catalogue No. 124031)

Activation of the Akt pathway can mediate evasion of apoptosis by tumor cells, because Akt can both inactivate pro-apoptotic factors as well as activate transcription of survival genes. The InhibitorSelect Akt/PI 3-K/mTOR Pathway Panel consists of 12 highly potent and selective kinase inhibitors and a negative control for studying the Akt pathway in one convenient package to help elucidate specific steps in apoptosis.



Muse™ Caspase-3/7 Assay Kit

(Catalogue No. MCH100108)

This kit allows for the facile and rapid quantitation of cells in various stages of apoptosis by measuring two important parameters simultaneously: (1) Caspase-3/7 activation and (2) plasma membrane permeabilization and cell death. The kit employs a novel reagent, NucView™, for the detection of Caspase-3/7 activity and a cell death dye that reports membrane integrity or cell death. The assay provides relative percentage of both adherent and suspension cells that are live, in the early and late stages of apoptosis, and dead. Performed on the sophisticated yet simple Muse™ Cell Analyzer, this no-wash, mix and read assay requires minimal sample preparation to obtain accurate and precise results.

Key Products for Apoptosis

Antibodies and Proteins

Description	Catalogue No.
Anti-Cytochrome C, clone 7H8.2C12	MAB1800
Anti-Bax, N-terminus	06-499
Anti-Bcl2, clone 100	05-729
Caspase-3, Human, Recombinant, E. coli	235417

For our entire line of apoptosis, cell death and autophagy antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
ApopNexin™ Annexin V FITC Apoptosis Kit	APT750
TUNEL Apoptosis Detection Kit	17-141
MILLIPLEX® MAP Human Akt/mTOR 11-Plex, Phosphoprotein	48-611MAG
MILLIPLEX® MAP Human Akt/mTOR 11-Plex, Total Protein	48-612MAG
Muse™ Annexin V & Dead Cell Assay Kit	MCH100105
Muse™ Caspase-3/7 Assay Kit	MCH100108
Muse™ Mitopotential Assay Kit	MCH100110
Muse™ MultiCaspase Assay Kit	MCH100109
FlowCellect™ MitoLive Kit	FCCH100107
FlowCellect™ MitoStress Kit	FCCH100109
FlowCellect™ Cytochrome c Kit	FCCH100110

Inhibitors

Description	Catalogue No.
InhibitorSelect™ EGFR Signaling Pathway Panel	324839
InhibitorSelect™ MAPK Signaling Pathway Panel	444189
InhibitorSelect™ NF-kB Signaling Pathway Panel	481487
InhibitorSelect™ JAK/STAT Signaling Pathway Panel	420138
InhibitorSelect™ WNT Signaling Pathway Panel	681666

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

Apoptosis Cell Based Assays From Merck Millipore

EARLY STAGE

LATE STAGE

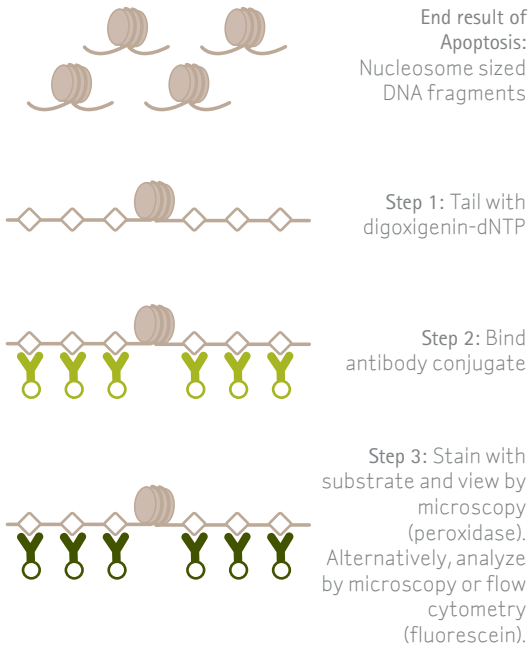
Hallmark Apoptotic Event	Concept	Merck Millipore Product	Detection Options	Sample Type
Phosphatidylserine Detection (PS)	Detection of early Apoptosis with direct staining of the phosphatidylserine flip on the outer cell membrane	ApopNexin™ Kit	Fluorometric	Adherent cells
				Suspension cells
		Guava Nexin® Reagents	Fluorometric	Suspension cells
		Anti-Phosphatidylserine	Colorimetric	Adherent cells
			Fluorometric	Suspension cells
Mitochondrial Permeabilization	Direct measure of changes in mitochondrial membrane potential	Guava® Mitochodrial Depolorization Kit	Fluorometric	Suspension cells
	Detect potential-sensitive color shifts induced by uncouplers of mitochondrial respiration	MitoLight® Mitochondrial Apoptosis Detection Kits	Fluorometric	Adherent cells
				Suspension cells
		Jc-1 dye	Fluorescent	Cell Lysates
Chromatin Condensation	Direct measure of accumulated phosphorylated H2A.X, an indicator of early DNA strand breaks	Phosphorylation Detection Kits	Colorimetric	Adherent cells
			Fluorometric	Suspension cells
			Chemiluminescent	Tissue
				Cell Lysates
	Formamide denaturation of DNA in apoptotic cells, but not in necrotic cells; reflects chromatin condensation	ssDNA ELISA Kit	Colorimetric	Adherent cells
		MILLIPLEX® MAP H2A.X MAPmate™	Fluorescent	Suspension cells
				Tissue
Caspase Cascade	Inverse measurement of specific caspase activity in live cells using inhibitor binding	CaspaTag™ Kits	Fluorometric	Adherent cells
				Suspension cells
				Tissue
	Direct measurement of **any** caspase activity in live cells following cleavage of a substrate	CaspSCREEN™ Kit	Fluorometric	Suspension cells
	Inverse measurement of specific caspase activity in live cells using inhibitor binding	Guava® Caspase and Dual Caspase Kits	Fluorometric	Suspension cells
	Direct measurement of specific caspase activity following cleavage of a substrate	Caspase Activity Kits	Colorimetric	Cell lysates
			Fluorometric	
			Chemiluminescent	
DNA Fragmentation	Detect DNA fragmentation using TUNEL methodology	Guava® TUNEL Kit	Fluorometric	Suspension cells
		ApopTag® TUNEL Kits	Colorimetric	Adherent cells
			Fluorometric	Suspension cells
				Tissue
	Identification of characteristic DNA fragmentation specific to apoptotic cells using oligonucleotide ligation	ApopTag® ISOL Kits	Colorimetric	Adherent cells
			Fluorometric	Suspension cells
				Tissue
	Direct measure of fragmented genomic DNA by electrophoretic separation	DNA Ladder Kit	Agarose	Cell lysates

Application	Advantages	Catalogue No.
Immunofluorescence	**Effective:** Classic model	APT750
Cytometry	**Versatile:** Detection options	
Flow Cytometry	**Effective:** Classic model	4500-0450, 4500-0455
	Versatile: Detection options	
Immunohistochemistry	**Sensitive:** Highly specific marker	05-719, 16-256
Immunofluorescence	**Versatile:** Detection options	
Cytometry		
Flow Cytometry	**Simple:** Results in 20 minutes	4500-0250
	Effective: Classic model	
Immunofluorescence	**Simple:** Results in 20 minutes	APT142, APT242
Cytometry	**Effective:** Classic model	
Multiplex	**Sensitive:** Highly specific marker	420200-5MG
Immunohistochemistry	**Sensitive:** Highly specific marker	17-327, 17-344
Immunofluorescence	**Flexible:** Multiple kit options	FCCS100182, FCCS025153, 05-636, 07-1590, 07-745, 09-018, 16-193, 16-202A, MABE171, and others
Flow Cytometry		
Immunofluorescence and Western Blot		
ELISA	**Sensitive:** Distinguishes apoptosis and necrosis with unique ssDNA antibody	APT225, 46-692
	Simple: Easy model to detect early apoptotic events	
Immunofluorescence	**High affinity:** Irreversible binding	APT400, APT403, APT408, APT409, APT420, APT423, APT428, APT429, APT500, APT523
Cytometry	**Effective:** Classic model	
Plate reader		
Cytometry	**Efficient:** Quick, direct measure of any caspase activation	APT105
Flow Cytometry	**High affinity:** Irreversible binding	4500-0500, 4500-0520, 4500-0530, 4500-0540, 4500-0550, 4500-0560, 4500-0570, 4500-0580, 4500-0590, 4500-0630, 4500-0640, 4500-0650
	Effective: Classic model	
Plate reader	**Versatile:** Fluorometric versus colorimetric options	APT129, APT131, APT163, APT165, APT166, APT168, APT169, APT171, APT172, APT173, APT176
Flow Cytometry	**Reliable:** Many peer-reviewed citations	4500-0121
	Sensitive: Low background with plant- derived labeling	
	Flexible: Multiple detection options	
	Accurate: Control slides available	
Immunohistochemistry	**Reliable:** Many peer-reviewed citations	S7100, S7101, S7110, S7111, S7160, S7165
Immunofluorescence	**Sensitive:** Low background with plant- derived labeling	
Cytometry	**Flexible:** Multiple detection options	
	Accurate: Control slides available	
Immunohistochemistry	**Sensitive:** Minimize background with *in situ* labeling technique	APT1000, S7200
Immunofluorescence	**Accurate:** Reduction of false positives	
	Unique: Differentiation of Type I (caspase independent) and Type II (caspase dependent) events	
Electrophoresis	**Effective:** Classic model	APT151
	Inexpensive: No analysis equipment	
	Fast: Less than 90 minutes	

Family of TUNEL Assay Products for Apoptosis Detection

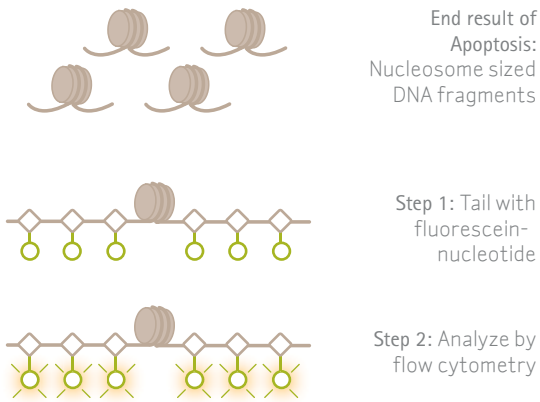
DNA fragmentation is usually associated with ultrastructural changes in cellular morphology in apoptosis. The ApopTag™ family of kits examines apoptosis via DNA fragmentation by the TUNEL assay. The DNA strand breaks are detected by enzymatically labeling the free 3'-OH termini with modified nucleotides. 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.

Indirect



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™ Caspase 3,7 *In Situ* Assay Kit, Sulforhodamine	APT523

Direct



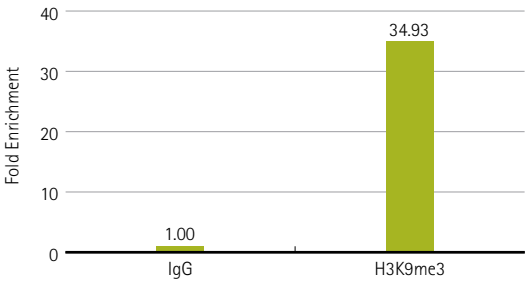
Limitless Replicative Potential

In normal cells, telomeres protect chromosomes from fusing with each other or rearranging during mitosis. Telomeres become shorter with each cell division, limiting cells to a fixed number of divisions. Tumor cells can achieve unlimited replicative potential either by synthesizing high levels of telomerase enzyme or via homologous recombination to create lengthened telomeres. Upregulation of telomere maintenance occurs in about 90% of human cancers. Increasing evidence indicates that chromatin modifications are important regulators of telomeres. Loss of epigenetic regulation correlates with aberrant telomere length. These links between epigenetic status and telomere-length regulation provide important insights for analyzing cancer progression and aging.

ChIPAb+™ Trimethyl-Histone H3 (Lys9)

(Catalogue No. 17-625)

Telomeres are normally enriched in epigenetic marks that are characteristic of heterochromatin, such as di- and trimethylated H3K9 (H3K9me3) and H4K20. These marks, along with phosphorylation of H3 (Ser10) and others, are correlated with chromatin condensation and active replication. In contrast, epigenetic marks such as phospho-H2A.X (Ser139) and phospho-H2B (Ser14) mark active apoptosis and DNA damage. The ChIPAb+™ validated antibody/primer set for chromatin immunoprecipitation of H3K9me3 complements Merck Millipore's broad range of specific, highly validated antibodies and assays for epigenetic signaling pathways.

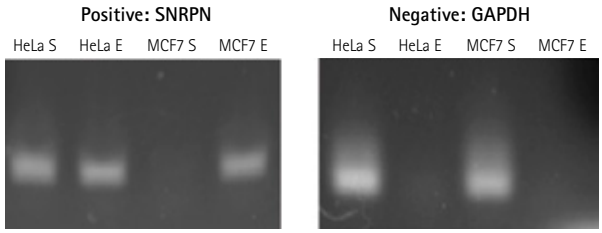


Sonicated chromatin prepared from 3x106 NIH 3T3 L1 cells was subjected to chromatin immunoprecipitation using 4 µg of either normal rabbit IgG or Anti-trimethyl-Histone H3 (Lys9) antibody (17-625) and the Magna ChIP A kit (17-610). Successful enrichment of trimethyl-histone H3 (Lys9)-associated DNA fragments was verified by qPCR using included ChIP primers flanking the mouse p16 promoter.

CpG MethylQuest™ DNA Isolation Kit

(Catalogue No. 17-10035)

Aberrant hypermethylation of normally unmethylated CpG islands is a frequent event in immortalized and transformed cells, and is associated with transcriptional inactivation of tumor suppressor genes. DNA methylation also correlates with repressed telomere recombination. Merck Millipore's CpG MethylQuest™ DNA Isolation Kit provides an efficient, convenient solution for mapping methylation across a single gene or across the entire genome, using a unique GST-methyl-CpG binding domain fusion protein that tightly binds CpG methylated sequences but has extremely low affinity for non-methylated regions.

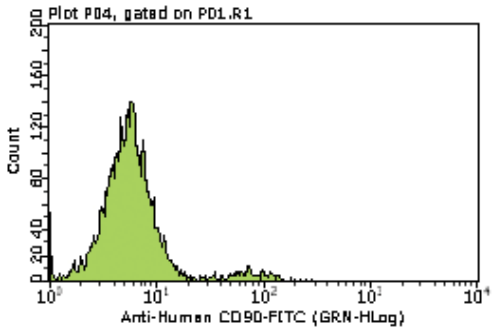


HeLa and MCF7 genomic DNA were purified with the CpG MethylQuest. DNA isolation kit. 2 µL of the purified samples were used for 30 cycles of PCR amplification of SNRPN (frequently methylated locus) and GAPDH (typically unmethylated). PCR aliquots were loaded on agarose gels (S = supernatant, not bound to beads; E = elution, bound to beads)

Fluorescent Conjugated Antibodies for Cell Surface CD Markers

(Catalogue Nos. FCMAB211F, FCMAB188F and others)

The replicative potential of cancer cells is analogous to that of stem cells. In fact, many studies have shown tumors' stem-like characteristics to correlate with malignancy. One strategy to isolate cancer cells with high replicative potential is using flow cytometry to identify cells that express stem cell surface markers, such as CD90 and CD34. Merck Millipore's growing selection of directly conjugated antibodies for cancer-relevant CD markers is specifically designed, optimized, and validated for flow cytometry.



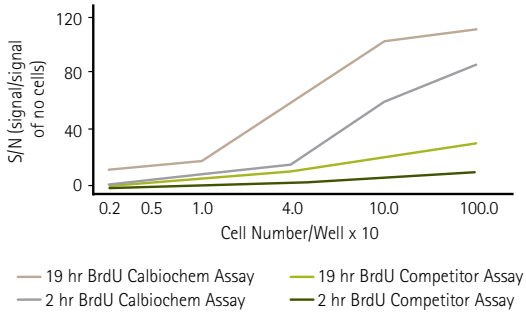
Human peripheral blood lymphocytes were stained with Milli-Mark™ human CD90-FITC antibody and analyzed by flow cytometry. The right-hand (lower) peak represents CD90-expressing cells.

Calbiochem® BrdU Cell Proliferation Assay

(Catalogue No. QIA58)

DNA replication in normal and tumor cells can be accurately measured by assessing the degree to which BrdU, a modified nucleotide, is incorporated into DNA. This BrdU Cell Proliferation Assay is a non-isotopic immunoassay for quantification of BrdU incorporation into newly synthesized DNA of actively replicating genomes. It is sensitive, rapid, and easy to perform.

Sensitivity of Calbiochem BrdU Cell Proliferation Assay vs. Competitor BrdU Labeling Kit



The Calbiochem® BrdU Cell Proliferation Assay responds to increasing cell number with higher sensitivity than a competing assay.

Key Products for Replicative Potential

Antibodies and Proteins

Description	Catalogue No.
Anti-phospho-Histone H2A.X (Ser139), clone JBW301	05-636
Anti-phospho-Histone H2B (Ser14)	07-191
Anti-HDAC9, clone LH/JC2	05-897
Aurora A, active purified kinase	14-511
Anti-CD34 Class III, clone 581, FITC conjugated	CBL555F

For our entire line of nuclear signaling and epigenetics antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
CpGenome™ Turbo Bisulfite Modification Kit	S7847
CpG WIZ® ERa Amplification Kit	S7815
TRAPeze® Telomerase Detection Kit	S7700
BrdU Immunohistochemistry System	HCS30
Senescence Detection Kit	QIA117
Telomerase Inhibitor III, Sodium Salt	581004
Telomerase Inhibitor IX	581011
PIPER	528120

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

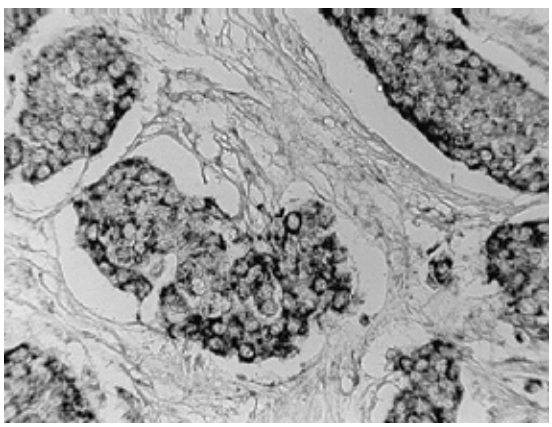
Sustained Angiogenesis

Angiogenesis, the development of new vascular networks, is rare in adult tissues. In tumors, dysregulated signaling and hypoxic conditions lead to sustained, almost uncontrolled angiogenesis, a necessary component of tumor growth and metastasis. Chronic inflammation mechanisms, such as the production of reactive oxygen species during infection and secretion of pro-inflammatory cytokines, can also foster angiogenesis in tumor progression. Angiogenic signaling in tumors is similar to normal angiogenesis, mediated by soluble growth factors, membrane-bound receptors, and cell-cell and cell-matrix interactions. Such signaling regulates cell migration, which is vital to angiogenesis. However, there are multiple differences between tumor angiogenesis and normal blood vessel formation. Tumor endothelial cells proliferate faster than non-tumor endothelial cells. Tumor vasculature differs from normal vasculature in morphology, enhanced leakiness, and structural abnormalities. Finally, tumor vessels are often not capable of transporting oxygen to and removing waste products from all of the tumor tissues, resulting in frequent tumor cell necrosis.

Anti-VEGF, clone JH

(Catalogue No. 05-443)

Vascular endothelial growth factor (VEGF) is a pro-angiogenic growth factor both secreted by tumor cells and expressed in response to hypoxia via HIF signaling. Specially validated for immunohistochemistry of paraffin-embedded tissues, this antibody complements Merck Millipore's comprehensive offering of antibodies against targets of various stimulating mechanisms of angiogenesis, including various VEGF isoforms, VEGF Receptors, FGFs, and angiopoietins.

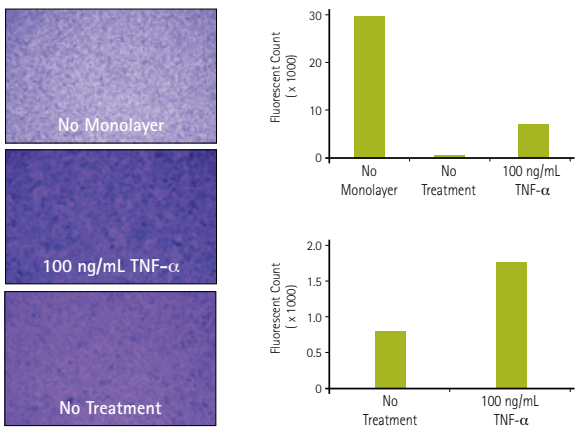


Immunohistochemistry of paraffin-embedded human breast carcinoma stained with anti-VEGF (05-443).

In Vitro Vascular Permeability Assays (24- & 96-Well)

(Catalogue Nos. ECM644, ECM642)

The *In Vitro* Vascular Permeability Assay provides an efficient system for evaluating the effects of chemicals and drugs on endothelial cell adsorption, transport, and permeability. Endothelial cells are seeded onto collagen-coated inserts in multiwell plates and treated with cytokines, growth factors, or any reagent of interest. After treatment, the extent of permeability is determined by measuring the fluorescence of the plate well solution.



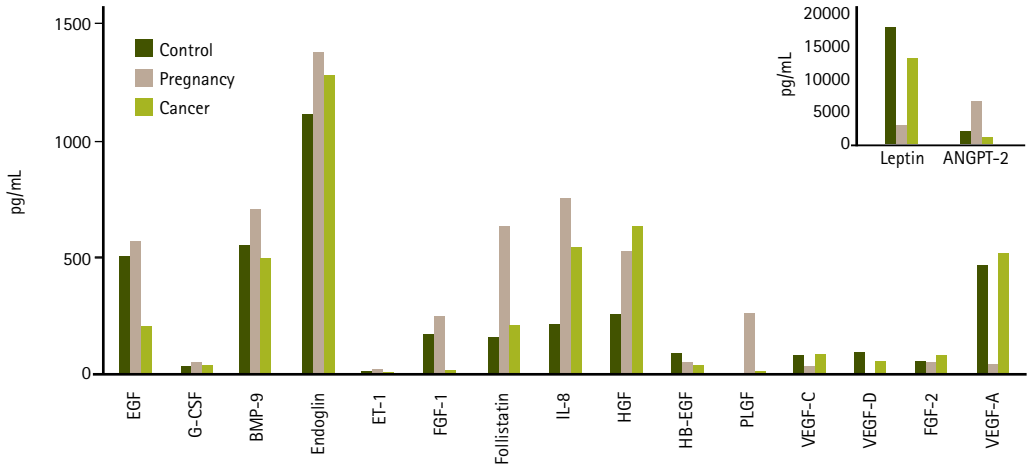
Permeability analysis of HUVEC monolayers. HUVEC cells were cultured, treated with TNFa, then subjected to FITC-dextran permeability testing and monolayer staining. Data showed high permeability in the absence of an occlusive endothelial cell monolayer. The "No Treatment" sample exhibits a visually confluent monolayer, as supported by low FITC-dextran permeability. Disruption of monolayer integrity is observable both visually and by quantification of increased plate well solution fluorescence following TNFa treatment.

MILLIPLEX® Human Angiogenesis/Growth Factor Panel

(Catalogue No. HCYTOMAG-60K)

The process of angiogenesis is normally a tightly regulated balance between proangiogenic growth factors and antiangiogenic signaling molecules, along with complex membrane-bound receptors and endothelial cell interactions. Because of the complexity of the angiogenesis pathway, the measurement of individual vascular and endothelial biomarkers is often inadequate to fully characterize the angiogenesis process in both normal and diseased states. Multiplexed detection of these analytes not only conserves precious samples but can also lead to a clearer picture of tumor progression.

Serum samples obtained commercially were analyzed using the MILLIPLEX® MAP Human Angiogenesis/Growth Factor Panel protocol. Each mean value (calculated using duplicate wells) observed from normal control subjects (n = 8), pregnant subjects (n=5) and patients with various tumor types (n= 35) is displayed.



Key Products for Angiogenesis

Antibodies and Proteins

Description	Catalogue No.
Anti-Angiopoietin-1	AB10516
Anti-VEGF Receptor-3, extracellular domain, clone 9D9F9	MAB3757
Anti-FGF-2/basic FGF (neutralizing), clone bFM-1	05-117

For our entire line of angiogenesis antibodies for cancer, search: **www.millipore.com/antibodies**

Related Inhibitors

Description	Catalogue No.
InhibitorSelect™ VEGF Signaling Pathway Panel	676502
TAS-301	608050
VEGF Inhibitor, CBO-P11	676496
Withaferin A, Withania somnifera	681535

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

Assays

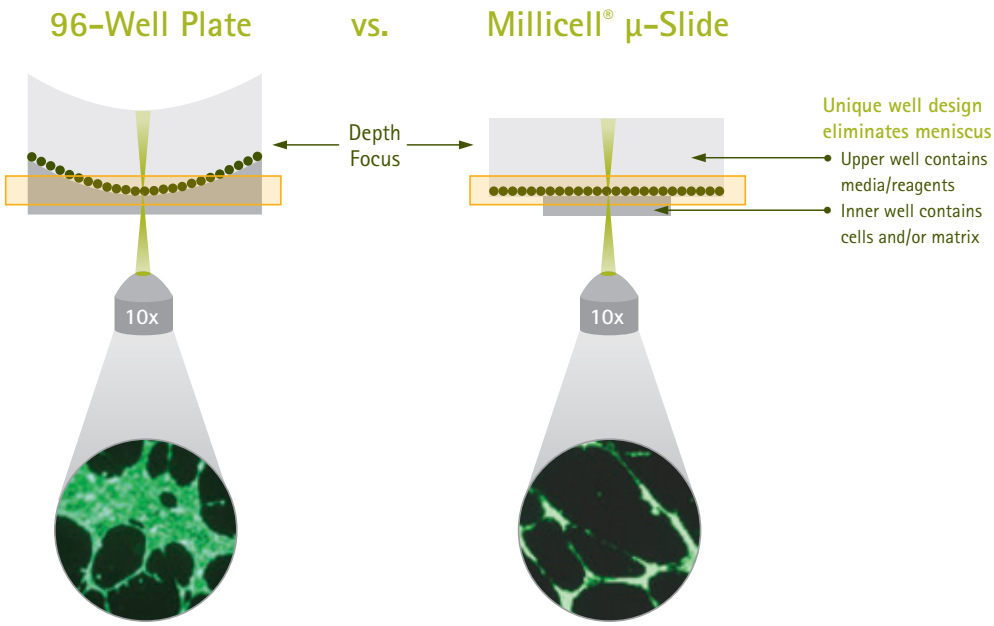
Description	Catalogue No.
In Vitro Angiogenesis Assay Kit	ECM625
Fibrin *In Vitro* Angiogenesis Assay	ECM630
MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel I	HCYTOMAG-60K
MILLIPLEX® MAP Human Soluble Cytokine Panel	HSCR-32K
ChemiScreen™ CXCR2 Chemokine Receptor Membrane Preps	HTS002M
Ready-to-Assay™ EP2 Prostanoid Receptor Frozen Cells	HTS185RTA

Millicell® µ-Angiogenesis Assay Kits

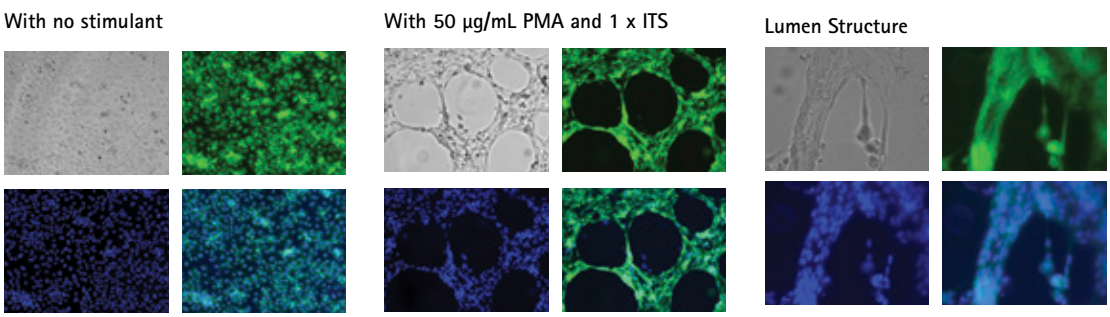
(Catalogue Nos. MMA125 and MMA130)

Quantify activation or inhibition of tumor angiogenesis with Millicell® µ-Angiogenesis Assay Kits. These kits provide a powerful, quantitative, slide-based platform for superior, real-time visualization of tubule formation in angiogenesis. The kits are optimized and easy to use with properties that prevent meniscus formation and out-of-focus areas. An entire well can be visualized at low magnification, generating enormous time and cost savings.

Enhanced imaging capabilities of Millicell® slides improve studies of angiogenesis:



Grow, treat, stain, and visualize cells all in one device:



Millicell® µ-Angiogenesis Activation Assay. The angiogenesis activation kit includes fibrinogen and thrombin components to make the fibrin gel, the activation control PMA (phorbol myristate acetate), and calcein-AM to visualize the cells in real time. (Left to right and top to bottom) Bright field, calcein-AM, DAPI, and calcein-DAPI merge micrographs show robust tubule formation and lumen structure in the presence of phorbol myristate acetate (PMA, a proangiogenic agent).

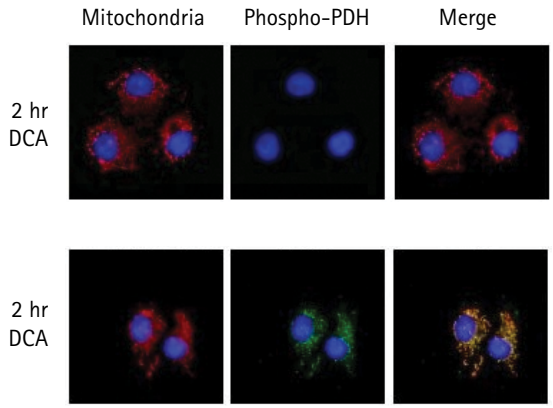
Metabolic Reprogramming

Normal cellular metabolism involves a complex series of controlled biochemical reactions that produce energy in the form of ATP to maintain homeostasis and allow cells to respond to environmental changes. These biochemical reactions are disrupted during tumorigenesis as cancer cell proliferation results in increased distances from vascular basement membranes, causing regional hypoxia. When ATP production from glucose metabolism falls below maintenance levels, there is an upregulation of anaerobic glycolysis that can then become permanent through the stabilization of hypoxia-inducible factor 1a (HIF-1a) and/or the upregulation of phosphorylated c-myc. This glycolytic adaptation causes regional acidosis, which provides growth advantages to the cancer population, allowing tumor cells to modify their environment in a way that is toxic to non-tumor cells. The acidic environment also leads to increased motility and cancer cell invasion into adjacent normal tissue, putting cancer cells in direct contact with mesenchymal cells.

Anti-PDH-E1a (pSer293) Rabbit pAb

(Catalogue No. AP1062)

Dysregulation of the pyruvate dehydrogenase (PDH) complex (PDC) in skeletal muscle has been implicated in type 2 diabetes and various mitochondrial diseases. Recently, it has been shown that inhibition of PDC activity in cancer cells promotes Warburg metabolic and malignant phenotype. The activity of PDC is mainly regulated by the phosphorylation state of Ser293, Ser232, and Ser300 on the E1a subunit.



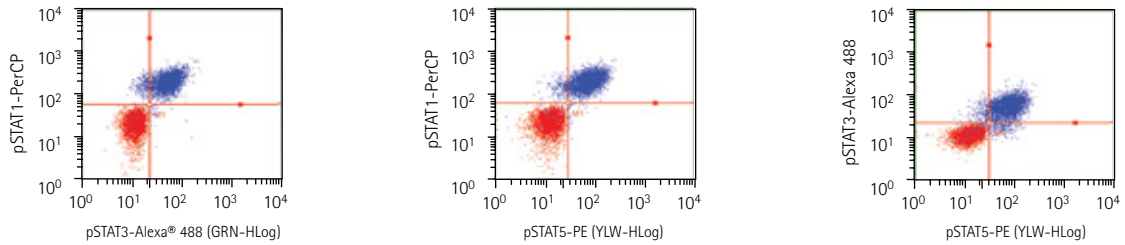
Detection of phospho-PDH-E1a (Ser293) by immunocytochemistry. COS7 cells were incubated in dichloroacetate (DCA), an inhibitor of PDH kinase. All samples were incubated with mitochondrial stain (red), fixed and permeabilized. Primary antibodies: PhosphoDetect™ Anti-PDH-E1a (pSer293) Rabbit pAb (Catalogue No. AP1062, green, top) or anti-PDH-E1a 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.

FlowCellect™ Multi-STAT Activation Profiling Kit

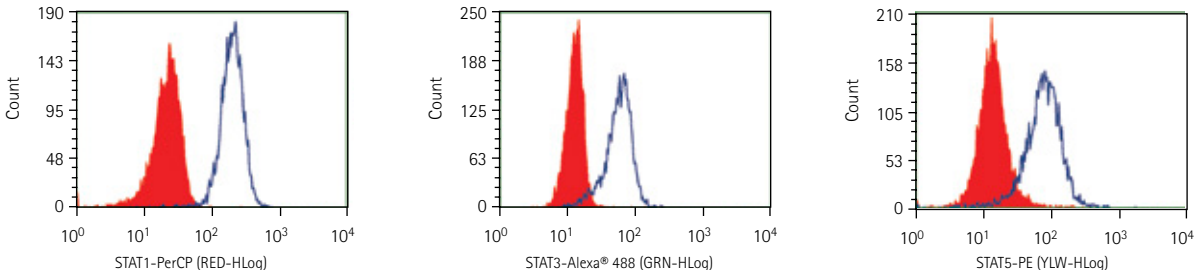
(Catalogue No. FCCS025550)

STAT transcription factors regulate cell proliferation, survival, and migration downstream of transmembrane receptors. Recent research reports additional roles for STATs in metabolic reprogramming. Active, nuclear STAT3 increases transcription of HIF-1a, promoting tumorigenesis, while mitochondrial STAT3 helps mediate transformation by affecting glycolysis and oxidative phosphorylation. By providing simultaneous detection of phosphorylated STATs, the FlowCellect™ Multi-STAT Activation Kit enables quick profiling of the status of constitutive activation of STAT1, STAT3, and STAT5 within a population of cells or tumors.

A.



B.

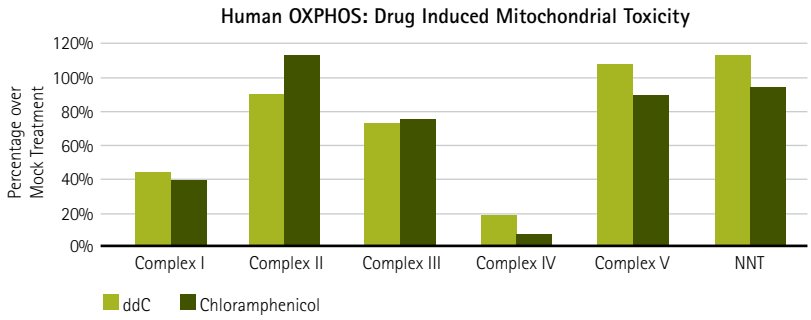


A. U937 cells were stimulated with IFNg, IL-6, and GMCSF, then stained with antibodies against phospho-STAT1, phospho-STAT3, and phospho-STAT5A/B. Double positive cells (blue) are seen in each plot, indicating simultaneous detection of all three activated STAT proteins. Untreated U937 cells (red) are shown for comparison. B. Single parameter overlays of activated STAT proteins in untreated (red) vs. treated (blue) U937 cells stimulated with IFNγ for STAT1 activation, IL-6 for STAT3 activation, and GM-CSF for STAT5A/B activation.

MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel

(Catalogue No. H0XPSMAG-16K)

Mitochondrial OXPHOS produces more than 95% of the conserved cellular energy in the form of ATP under normal conditions. Without proper OXPHOS regulation, the production of toxic reactive oxygen species is known to increase, potentially leading to tumor development. The MILLIPLEX® MAP Human OXPHOS Magnetic Bead Panel uses the Luminex® xMAP® platform to simultaneously detect changes in the 5 OXPHOS multiprotein complexes, plus NNT as an internal reference for mitochondrial proteins.



Human OXPHOS changes upon drug induced mitochondrial toxicity. Human HepG2 cells were treated with chloramphenicol (an antibiotic), 2'-3'-dideoxycytidine (ddC, an antiviral drug), or DMSO (mock treatment). The cell lysates were prepared according the Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel protocol. Drug-induced mitochondrial toxicity was evaluated by normalizing the quantity of each analyte to mock-treated cells and reporting the results as percentages of the mock-treated values. Chloramphenicol and ddC clearly induced mitochondrial toxicity as demonstrated by the significant reduction of Complex I and IV while Complex II, V and NNT remained relatively unchanged.

Autophagy

Autophagy is an intracellular catabolic pathway which causes cellular protein and organelle turnover, and is associated with diverse diseases such as Alzheimer's disease, cancer, and Crohn's disease, in addition to aging. It is a tightly regulated process that plays a normal part in cell growth, development, and cellular homeostasis. Malfunctions of autophagy can adversely impact longevity and the capability of cells to function at full capacity. In cancer cells, autophagy can compensate for hypoxic conditions and nutrient starvation; on the other hand, activation of cell death via autophagy can kill tumor cells. As a result, there is great interest in assays that can efficiently screen for activators and inhibitors of autophagy.

Merck Millipore is the leader in providing new, innovative products for studying autophagy mechanisms and identifying activators and inhibitors of autophagy.

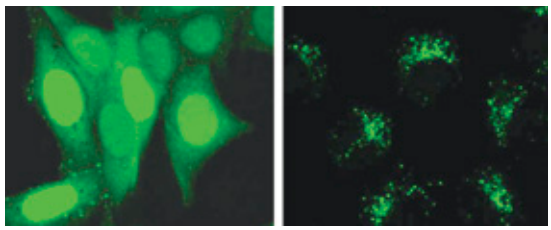
Lentiviral Biosensors for Live Cell Analysis of GFP- & RFP-LC3 and p62 Localization

Merck Millipore's new LentiBrite™ Lentiviral Biosensors, a new suite of pre-packaged lentiviral particles encoding the foundational protein of autophagy detection - LC3 and chaperone protein p62, enabling precise visualization of autophagosome formation under different cell/disease states in live or fixed cells. Visualize autophagy in real time, even in difficult-to-transfect cell types, using LentiBrite™ GFP- & RFP-tagged LC3 wild-type, p62-wild-type and LC3-G120A mutant control lentiviral biosensors.

LentiBrite™ Biosensor Advantages:

- Pre-packaged, ready-to-use, fluorescently-tagged LC3 and P62 with monomeric GFP & RFP
- Minimum titer (≥3 x 10^8 IFU/mL) per vial
- Long-term, stable fluorescent expression that is non-disruptive towards cellular function
- Higher efficiency transfection as compared to traditional chemical-based and other non-viral-based transfection methods
- Ability to transfect dividing, non-dividing, and difficult-to-transfect cell types, such as primary cells or stem cells
- Validated for fluorescent microscopy and live cell analysis
- LC3 Control Mutant lentiviral particle contains the translocation-defective protein LC3-G120A for comparison studies.

Uninduced | Induced



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

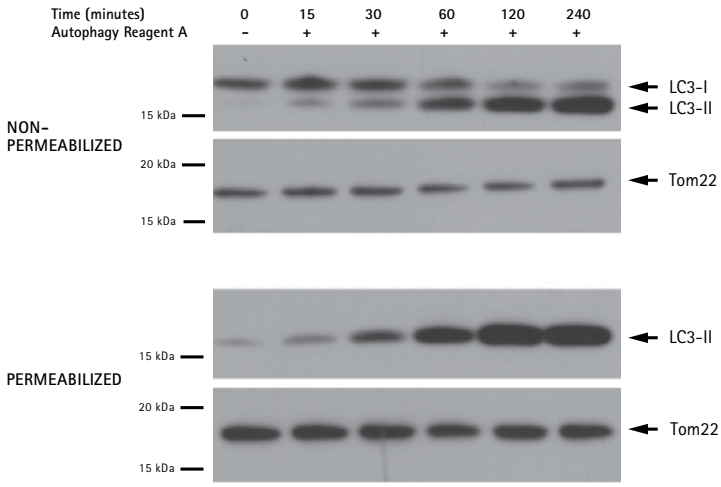
Description	Catalogue No.
LentiBrite™ GFP-LC3 Lentiviral Biosensor	17-10193
LentiBrite™ RFP-LC3 Lentiviral Biosensor	17-10143
LentiBrite™ GFP-LC3 Control Mutant Lentiviral Biosensor	17-10189
LentiBrite™ RFP-LC3 Control Mutant Lentiviral Biosensor	17-10188
LentiBrite™ GFP-p62 Lentiviral Biosensor	17-10224
LentiBrite™ RFP-p62 Lentiviral Biosensor	17-10404

LC3-II Enrichment Kit (Western Blot & Flow Cytometry)

(Catalogue No. 17-10232)

LC3 precursors are processed to form LC3-I, which is diffusely distributed in the cytosol. Upon initiation of autophagy, LC3-I is conjugated to phosphatidylethanolamine (PE) to form LC3-II, which translocates to autophagosomes. Detecting and interpreting the relative amounts of LC3-I and LC3-II in standard Western blots can be complicated; the LC3-I and LC3-II bands are sometimes incompletely resolved.

The LC3-II Enrichment Kit (Western Blot) enables sensitive and accurate quantification of autophagosome density. Using selective permeabilization, the kit removes cytosolic LC3-I and retains autophagosome-bound LC3-II. This procedure facilitates quantitation of LC3-II, without interference from LC3-I, by Western blotting.



Non-enriched lysate and LC3-II-enriched protein fraction from HeLa cells were prepared with the LC3-II Enrichment Kit (Western Blot, Catalogue No. 17-10232). Western blots of non-enriched lysates indicate the LC3-I signal decreases over time after induced autophagy, as the LC3-II signal increases. After enrichment, the LC3-I signal is no longer detectable and the LC3-II signal is retained.

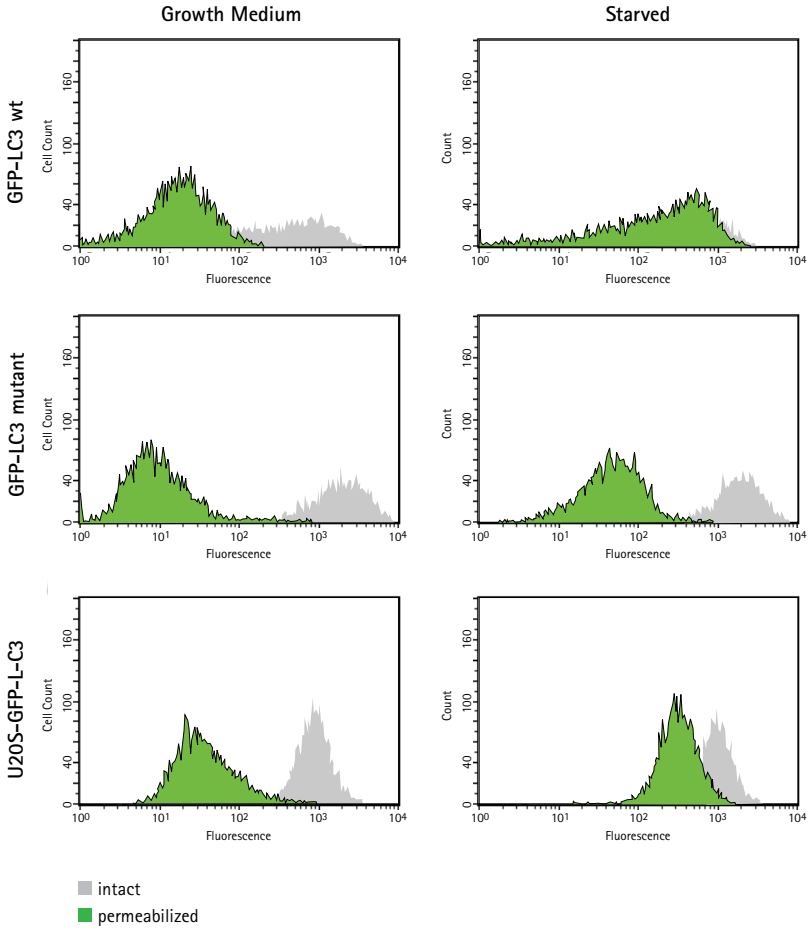
LentiBrite™ GFP-LC3-II Enrichment Kit (Flow Cytometry)

(Catalogue No. 17-10230)

Flow cytometry is a powerful means of assessing the extent of LC3-II localization to the autophagosome in individual cells. However, cytosolic LC3-I (non-lipidated form) can cause persistent background signal and can make quantitation difficult.

The LentiBrite™ LC3-II Enrichment Kit for flow cytometry selectively permeabilizes the plasma membrane, so that the cytosolic, fluorescent protein-tagged LC3 is released while autophagosome-bound LC3 fusion protein is retained.

Graphs (right): Analysis of GFP-LC3 localization in HUVEC by flow cytometry. HUVECs were lentivirally transduced with TagGFP2-LC3 wild-type (GFP-LC3 wt, top row) or TagGFP2-LC3G120A control mutant (GFP-LC3 mutant, center row). U2OS cells stably expressing TagGFP2-LC3 wild-type were also analyzed (U2OS-GFP-LC3, bottom row). Transduced cells were detached and either permeabilized to release free, cytosolic LC3 (green peaks) or left intact (gray peaks). After processing, the cells were analyzed by flow cytometry on a guava easyCyte™ 8HT instrument. Upon permeabilization, only TagGFP2-LC3 wild-type-expressing cells under starvation conditions display retention of the fusion protein, indicative of tight association of LC3 with autophagosomes.



Related Products

Description	Applications	Catalogue No.
Anti-LC3A, clone EPR1754	WB, FC	MABC175
Anti-LC3A/B (N-term), clone EP1983Y, Rabbit Monoclonal	WB, IHC, ICC, IF, IP, Flow	MABC176
Anti-LC3A (N-term), clone EP1528Y, Rabbit Monoclonal	WB, IHC, ICC, IP	MABC177
Anti-ATG3	WB	AB2953
Anti-ATG4C	WB	ABC21
Anti-ATG5	WB, IHC	ABC14
Anti-ATG5	WB, ICC, IHC	AB15404
Anti-ATG5	WB, ICC, IHC	AB15404P
Anti-ATG5, clone 177.19	WB, IP	MAB2605
Anti-ATG7	WB, IHC	AB10511
Anti-ATG7, clone EP1759Y	WB, ICC, IHC, IP, Flow	04-1055
Anti-ATG9 L2	WB, ICC, IHC	AB15407
Anti-ATG10	WB. ICC. IHC	AB15408
Anti-ATG12	WB, ICC, IHC	AB15410
Anti-ATG16L1	WB, IHC	ABC25
Anti-UVRAG	WB, ICC, IP	AB2960
Anti-SOGA	WB	ABS91
Anti-mTOR, clone 21A12.2	WB, ICC	05-1564
Anti-phospho-mTOR (Ser2448)	WB	09-213
Anti-Beclin-1, clone EPR1733Y	WB, ICC, IHC, IP	MABN16
Anti-Beclin-1	WB, ICC, IHC	AB15417

For our entire line of autophagy antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
FlowCellect™ GFP-LC3 Reporter Autophagy Assay Kit (CHO)	FCCH100170
FlowCellect™ GFP-LC3 Reporter Autophagy Assay Kit (U2OS)	FCCH100181
FlowCellect™ Autophagy Detection Reagent Pack, 100 tests	CF200097
FlowCellect™ Autophagy LC3 Antibody-based Assay Kit (100 tests)	FCCH100171

Small Molecule Modulators of Autophagy

Description	Product	Catalogue No.
Negative control for inhibition of EGFR	AG 9	658390
EGFR tyrosine kinase	AG 112	658440
Akt	Akt Inhibitor IV	124011
Akt1, Akt2, Akt3	Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	124018
Akt	Akt Inhibitor X	124020
PKC	Bisindolylmaleimide I	203290
DNA-PK, PI3-K, and mTOR	PI-103	528100
PI 3-Kγ	PI 3-Kγ Inhibitor	528106
PKCβII, βI	PKCß Inhibitor	539654
p70 S6 kinase	Rapamycin	553210
ROCK	Rho-Kinase Inhibitor III, Rockout	555553
PDGFRβ, VEGFR2, FGFR1, and Kit family members	SU11652	572660

Key Products for Metabolism

Antibodies and Proteins

Description	Catalogue No.
Anti-Hypoxia Inducible Factor 1a (HIF-1a), clone H1a67	MAB5382
Anti-Mitochondria, surface of intact mitochondria, clone 113-1	MAB1273
Anti-PI3 Kinase, p110a	09-481
Anti-PDH-E1a (pSer232) Rabbit pAb	AP1063
Anti-PDH-E1a (pSer300) Rabbit pAb	AP1064
Anti-BRCA1 (Ab-1) Mouse mAb (MS110)	OP92

For our entire line of metabolism antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
Mitochondria/Cytosol Fractionation Kit	MIT1000
MILLIPLEX® MAP Human Fatty Acid Oxidation Panel 1	HFA01MAG-11K
MILLIPLEX® MAP Multi-species Pyruvate Dehydrogenase (PDH) Complex	PDHMAG-13K
MILLIPLEX® MAP Total HIF-1a MAPmate™	46-665
FlowCellect™ EGFR/STAT1 Activation Dual Detection Kit	FCCS025142

Inhibitors

Description	Catalogue No.
HIF-1 Inhibitor	400083
HIF-1α/2α Inhibitor IV, FM19G11	400089

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

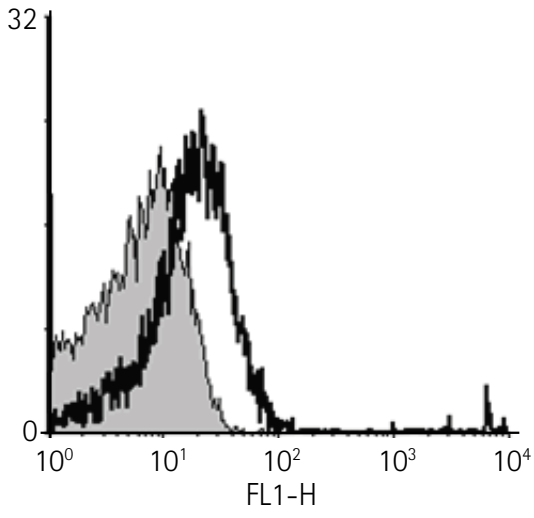
Escape From Immune Control

Unlike other harmful agents that invade the body, cancer cells avoid immune control by two major mechanisms: first, they avoid antigenic recognition by immune cells, and second, they neutralize immunogenic response. Cancer cells avoid antigenic recognition by hiding within difficult-to-access tissues and by shedding adhesion molecules and antigens that would otherwise attract immune cells. Cancer cells neutralize the immunogenic response by secreting immunosuppressive cytokines (such as TGFβ, IL-10, and VEGF) and by secreting anti-apoptotic signals. Cancer-driven suppression of immune response can potentially impact the way in which the host immune system recruits immune regulatory cells, uses inhibitory receptor-ligands, and produces inhibitory or inflammatory cytokines. Resulting dysfunctions in the host's own immune system further facilitate evasion of immune control by cancer cells.

Anti-FOXP3, clone 3G3

(Catalogue No. 04-960)

FOXP3 regulates the development and differentiation of T regulatory T-cells in the normal immune system. However, many cancers express high levels of FOXP3, suggesting that it mediates tumor escape from immune control. This FOXP3 antibody is validated for flow cytometry and ELISA, making it ideal for multiparametric studies of the immune system. It complements Merck Millipore's wide range of antibodies for studying immune cell regulation including antibodies against IL-17, CTLA4, various cytokines and chemokines, and members of the JAK/STAT pathway.

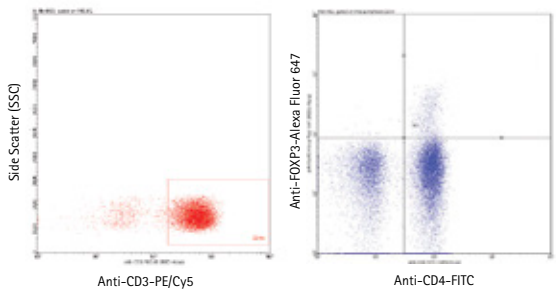


FoxP3 is expressed in mouse splenocytes. Surface staining of mouse CD4+ CD25+ splenocytes with 5 µg of a biotinylated version of Anti-FOXP3, clone 3G3 (open histogram) or with mouse biotinylated IgG isotype control (shaded histogram). Cells were detected using Streptavidin-FITC and analyzed using flow cytometry.

FlowCellect™ Human FOXP3 Treg Characterization Kit

(Catalogue No. FCIM025118)

Regulatory T-cells suppress the activation of the immune system following infections, maintaining homeostasis. Some cancer patients have increased numbers of regulatory T-cells, allowing malignant cells to escape the activation of the immune system. This kit helps phenotypically distinguish and quantify human regulatory T-cells with high accuracy and specificity. Our combination of directly conjugated antibodies and optimized buffers enable simultaneous detection of CD3 and CD4 on the surface and FOXP3 in the nucleus.



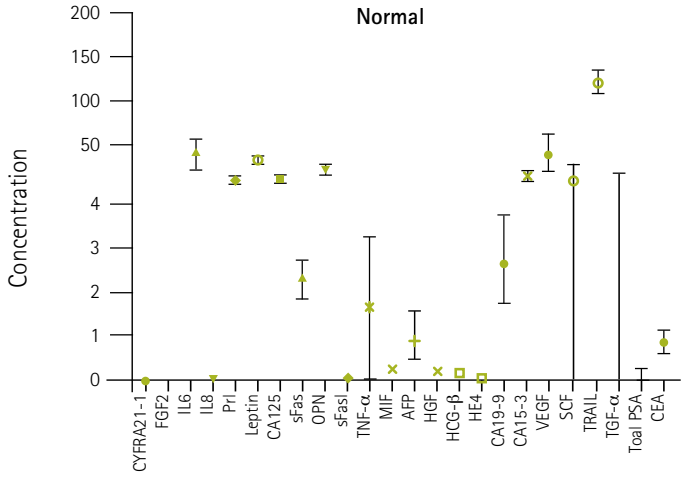
Human PBMCs were stimulated with IL-2 for 2 days. Total T-cells (CD3-positive lymphocytes) were analyzed for dual expression of CD4 and FOXP3 (shown in the upper right quadrant of right plot). 3.25% of the T-cells for this sample are CD3+, CD4+, and FOXP3+. A CD3 gate is used to eliminate cell types other than T-cells, such as monocytes, which can also express FOXP3, to avoid false positives.

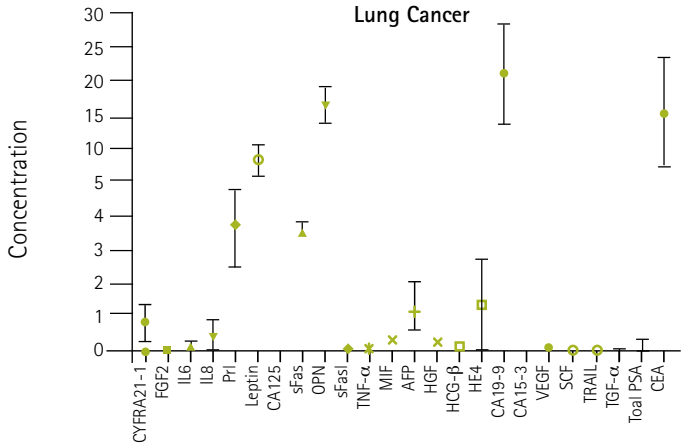
MILLIPLEX® MAP Human Circulating Cancer Biomarker Panel 1

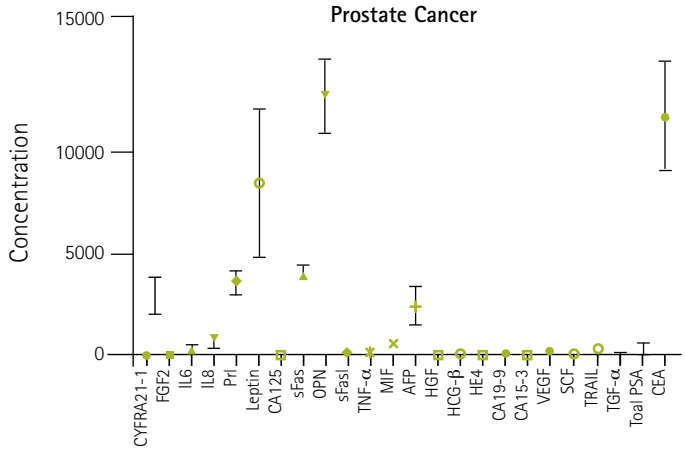
(Catalogue No. HCCBP1MAG-58K, magnetic)

Understanding the role of cytokines and chemokines secreted either by tumors to avoid immune control or by the body to effect immune response or inflammation has shed light on the interactions between a tumor and its environment. This multiplexed analyte panel consists of 26 circulating cancer biomarkers that include cytokines, chemokines, and metabolic markers. Some biomarkers are tumor type-specific, such as prostate-specific antigen (PSA), while others, such as IL-8, have been detected in many cancers.

Graphs (right): Detection of circulating cancer biomarkers using the MILLIPLEX® MAP Human Circulating Cancer Biomarker Panel 1 in normal, lung and prostate cancer samples.







Key Products for Immune Control

Antibodies

Description	Catalogue No.
Anti-CTLA4 (CD152), clone 9H10	04-963
Anti-JAK3, clone HL423	04-011
Milli-Mark™ Anti-Mouse IL-17 FITC Antibody, clone TC11-18H10.1	FCMAB255F
Anti-Macrophages/Granulocytes Antibody, clone OX-41	MAB1407P
Anti-Interleukin 21 (IL-21) Antibody, clone 1G8	04-1583

For our entire line of immunology, inflammation, cytokines, CD markers & innate immunity antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
FlowCellect™ Mouse FoxP3 Identification Kit	FCIM025126
FlowCellect™ Human Lymphocyte ZAP-70 Characterization Kit	FCIM025122
FlowCellect™ Mouse Th1/Th17 Intracellular Cytokine Kit	FCIM025138

Inhibitors

Description	Catalogue No.
InhibitorSelect™ JAK/STAT Signaling Pathway Inhibitor Panel	420138

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

Genome Instability & Mutation

Although some hallmarks, such as insensitivity to proliferation inhibiting signals, can be promoted by epigenetic changes to gene expression, most of cancer's hallmarks require changes in DNA sequence. These changes are caused by mutation or gene amplification. In normal cells, mutations are efficiently prevented by tumor suppressing systems that repair damaged DNA, inactivate DNA-damaging agents (such as reactive oxygen species) or promote genomic integrity. However, if any of these tumor suppressing mechanisms are disabled, the cell experiences increased mutation, chromosomal damage, recombination, and possibly aneuploidy. In a vicious cycle, these changes can lead to disablement of even more tumor suppressing mechanisms, in turn leading to the acquisition of more cancer hallmarks. Abundant evidence, gained largely from advances in high resolution tumor sequencing, has led to the addition of genome instability and mutation as a hallmark of cancer. Many cancers exhibit defects in p53 DNA repair pathway, telomerase-mediated chromosome protection, widespread genetic mutations and wildly varying gene copy numbers.

TRAPeze® Telomerase Detection Kits

(Catalogue Nos. S7700, S7707 and others)

Telomerase expression and telomere length stabilization are linked to extension of cell life span and tumor suppression. TRAPeze® telomerase detection kits are rapid, quantitative, *in vitro* assays for detecting activity. The original kit permits detection via PCR and gel electrophoresis. TRAPeze® telomerase detection kits are also available in colorimetric and fluorimetric formats as the TRAPeze® ELISA and TRAPeze® XL kits, incorporating biotinylated and fluorescent primers respectively.

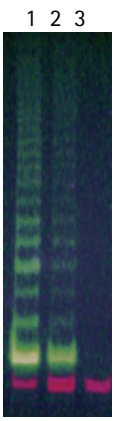
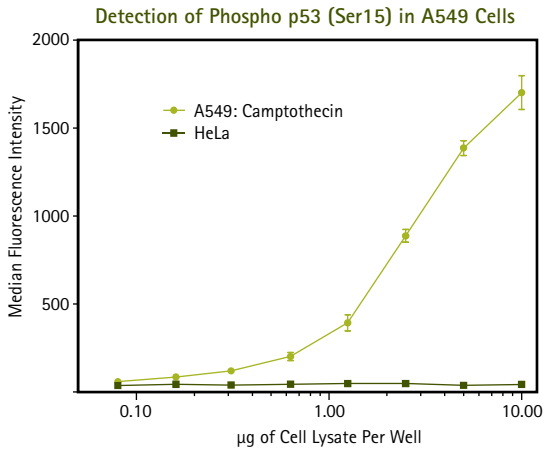


Image demonstrates the direct fluorescence imaging of the TRAPeze® XL reaction of three specimens – telomerase positive lanes 1 and 2, and telomerase negative lane 3.

MILLIPLEX® MAP Phospho-p53 (Ser15) MAPmate™

(Catalogue No. 46-663)

In response to DNA damage, p53 induces gene expression, such as for the Cdk inhibitor p21, that, in cooperation with p19ARF, causes cell cycle arrest. Inactivation or loss of p53 is associated with deregulation of the cell cycle and DNA replication, inefficient DNA repair, and the development of various human cancers. The MILLIPLEX® MAP Phospho p53 (Ser15) MAPmate™ pair can detect the presence of phosphorylated p53 (Ser15) in cell lysates using the Luminex® 100 IS, 200, or HTS system.

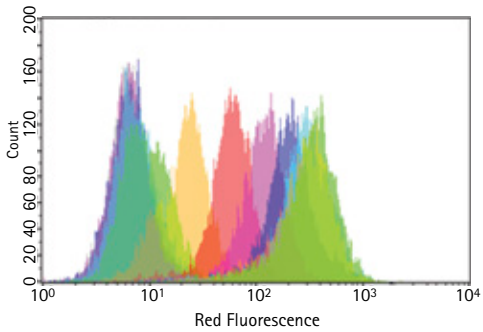


MILLIPLEX® MAP detection of phosphorylated p53 (Ser15) in A549 cell lysate. The Median Fluorescent Intensity (MFI) in triplicate wells was measured using the Luminex® Instrument. This graph displays the MFI values obtained utilizing the Phospho p53 (Ser15) MAPmate™.

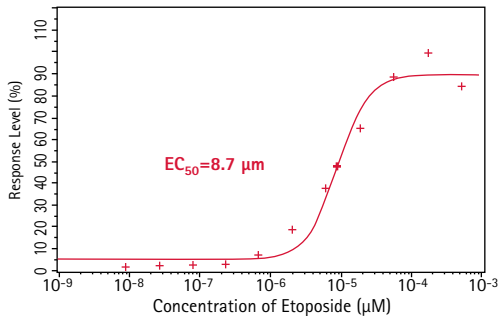
FlowCellect™ DNA Damage Histone H2A.X Dual Detection Kit

(Catalogue No. FCCS025153)

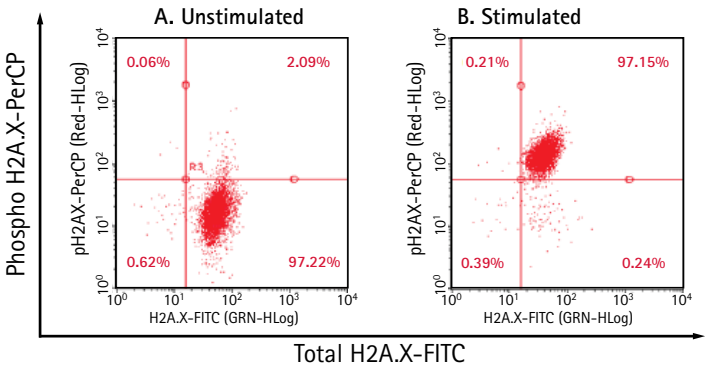
Phosphorylated H2A.X (Ser139) is the key component of the signal transduction pathways that are mobilized during DNA damage. The FlowCellect™ DNA Damage Histone H2A.X Dual Detection Kit uses pairs of total and phospho-specific antibodies for multicolor flow cytometry analysis. Simultaneous analysis of total and phosphorylated H2A.X will provide an accurate method to investigate levels of phospho-specific H2A.X and the degree of DNA damage.



InCyte™ software was used to generate overlaid histograms showing that increasing doses of etoposide resulted in increasing red fluorescence and increasing phosphorylation of H2A.X.



Using the curve-fitting functions of InCyte™ software, an EC_{50} of 8.7 µM was calculated for etoposide's effect on HeLa cells under the conditions of this experiment.



Dual Parameter Analysis of Total and Phospho Histone H2A.X on HeLa Cells. In untreated HeLa cells stained with both anti-phospho-Histone H2A.X-PerCP and Anti-Histone H2A.X-FITC (A), 97.2% of cells show positive signal for total H2A.X but no Histone H2A.X activation via phosphorylation. However, once HeLa cells were treated with 100 µM etoposide, 97.15% of the cells became positive for both total and phospho-H2A.X, confirming target specificity of the phosphorylation event (B). Only 2.09% of untreated cells were double positive (A).

Key Products for Genome Instability & Mutation

Antibodies and Proteins

Description	Catalogue No.
Anti-Chk1	04-207
Anti-Plk1	05-844
Anti-Wee1	06-972
Anti-TRF1, clone BED5 57-6	04-638
Anti-TRF2, clone 4A794	05-521

For our entire line of DNA damage and epigenetics antibodies for cancer, search: **www.millipore.com/antibodies**

Inhibitors

Description	Catalogue No.
PARP Inhibitor VIII, PJ34	528150
PARP Inhibitor Set	528820
ATM Kinase Inhibitor	118500
ATM/ATR Kinase Inhibitor	118501
InSolution™ ATM Kinase Inhibitor	118502

Assays

Description	Catalogue No.
H2A.X Phosphorylation Assay Kit, Flow Cytometry	17-344
FlowCellect™ Histone H2A.X Phosphorylation Assay Kit	FCCS100182
FlowCellect™ Cell Cycle Checkpoint H2A.X DNA Damage Kit	FCCH025142

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

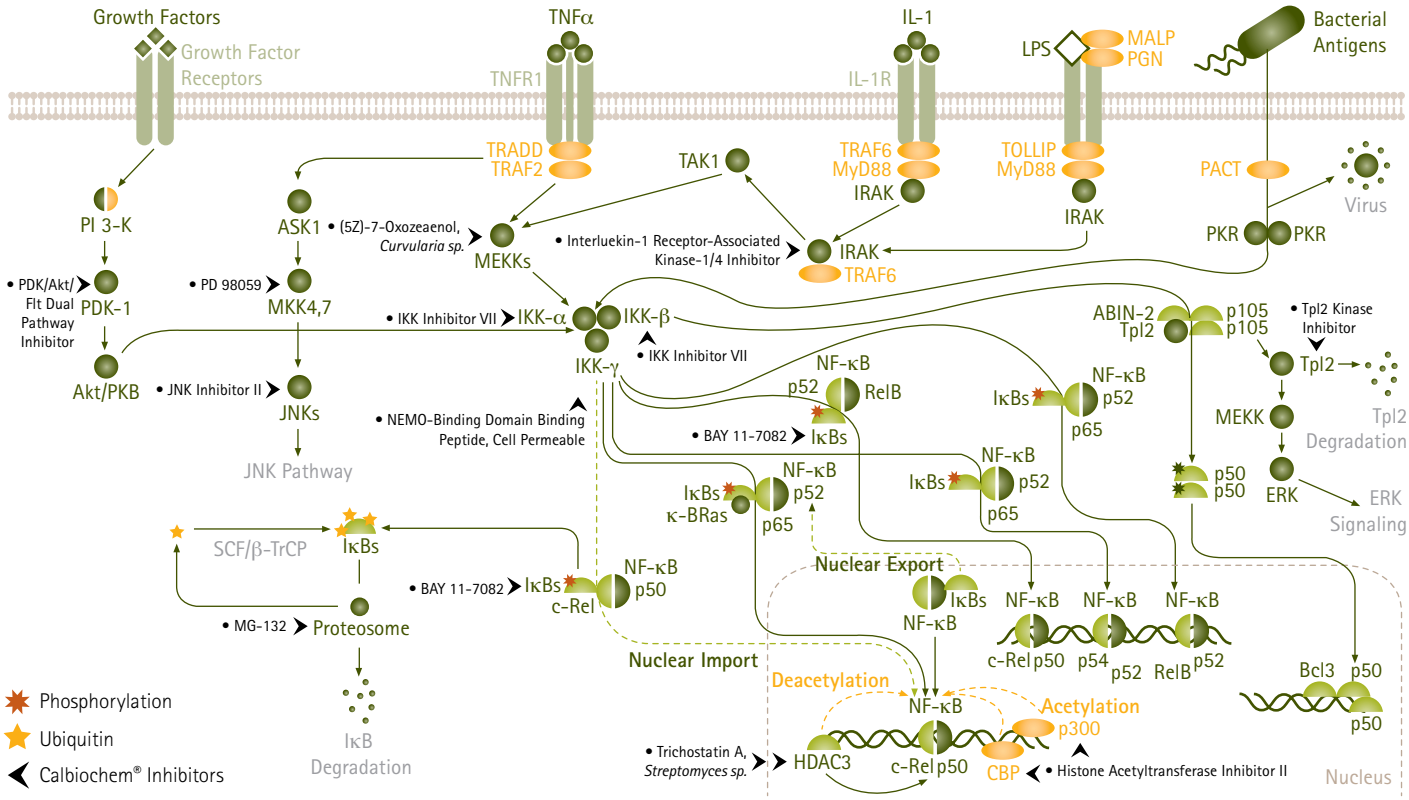
Tumor-Promoting Inflammation

Chronic inflammation mechanisms, such as the production of free radicals usually associated with infection, have been shown to foster tumorigenesis. In addition, inflammation in the tissues surrounding a tumor may result in immune cells penetrating into a tumor and releasing soluble signals that trigger the development of cancer hallmarks, such as sustained angiogenesis, aberrant cell proliferation and metastasis. These soluble factors include pro-inflammatory cytokines such as TGFβ, IL-6, IL-8 and TNF, modulators of the NFκB signaling pathway, proangiogenic factors, antiapoptotic signals, extracellular matrix-modifying enzymes and inflammation-induced growth factors. In particular, the TGFβ pathway has been the recent focus of studies exploring the link between inflammation and metastasis.

Calbiochem® InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel

(Catalogue No. 481487)

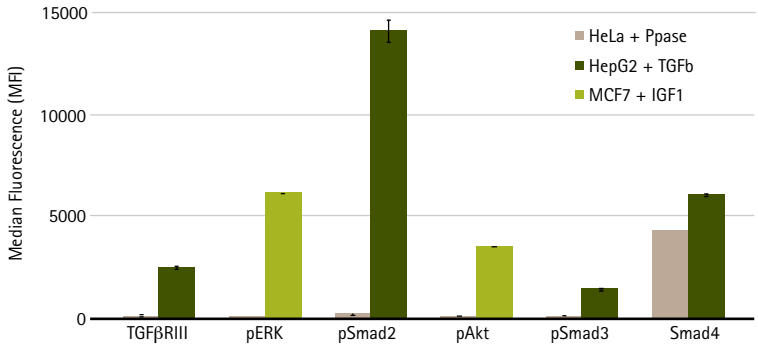
The eukaryotic nuclear factor κB (NF-κB) plays an important role in inflammation, autoimmune response, cell proliferation, and apoptosis by regulating the expression of genes involved in these processes. The Rel/NF-κB signal transduction pathway is mis-regulated in a variety of human cancers, especially those of lymphoid cell origin. Designing antitumor agents to block NF-κB activity or to increase their sensitivity to conventional chemotherapy may have great therapeutic value. Basing such target validation studies on a set of structurally diverse molecules such as in the InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel can provide a powerful starting point for developing therapeutics to inhibit tumor-promoting inflammation.



MILLIPLEX® MAP TGFβ Signaling Pathway Magnetic Bead 6-Plex

(Catalogue No. 48-614MAG)

TGFβ is a potential master regulator of tumor progression via induction of epithelial-to-mesenchymal transition. TGFβ is secreted by immune cells during tumor-associated inflammation, and initiates signaling that may enhance the migratory and invasive potential of target tumor cells. TGFβ signaling, however, is complex, and measuring one pathway protein at a time may be insufficient to unequivocally characterize signaling status. Use this multiplexed assay to quantify the levels of six TGFβ pathway proteins, including TGFβ receptor and several SMADs, to elucidate the link between proinflammatory signaling and cancer.



Multiplex analysis of six TGFβ signaling proteins in TGFβ-stimulated HepG2 cells and IGF1-stimulated MCF7 cells. HepG2 cells stimulated with 2 ng/mL of TGFβ (60 min) or MCF-7 cells stimulated with 50 ng/mL IGF-1 (10 min) were assayed. The cells were lysed in MILLIPLEX® MAP Lysis Buffer containing protease inhibitors. 20 µg total protein of each lysate diluted in MILLIPLEX® MAP Assay Buffer 2 were analyzed according the assay protocol (lysate incubation at 4 °C overnight). The Median Fluorescence Intensity (MFI) was measured with the Luminex® system. The error bars represent the standard deviation of three replicate wells.

Key Products for Tumor-Promoting Inflammation

Antibodies and Proteins

Description	Catalogue No.
Milli-Mark™ Anti-NFκB p52-FITC	FCMAB346F
Milli-Mark™ Anti-mouse CD44 (H-CAM)-PE, clone KM201	FCMAB426PE
Anti-STAT3 Antibody, clone E121-21	04-1014
PhosphoDetect™ Anti-STAT3 (pTyr705), clone 9E12	569384-1SET
Anti-phospho JAK-2 (Tyr813)	09-242

For our entire line of inflammation antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
NFκB p65 EZ-TFA Transcription Factor Assay	70-620
Mouse IL-6 ELISA Kit	EZMIL6
MILLIPLEX® MAP Total STAT1 MAPmate™	46-654

Inhibitors

Description	Catalogue No.
Non-steroidal Anti-inflammatory Drugs	
Celastrol, Celastrus scandens	219465
Meloxicam	444800
Cyclooxygenase (COX) Inhibitors	
COX-2 Inhibitor II	236012
COX-2 Inhibitor V, FK3311	236015
Cyclooxygenase Inhibitor Set	239783

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

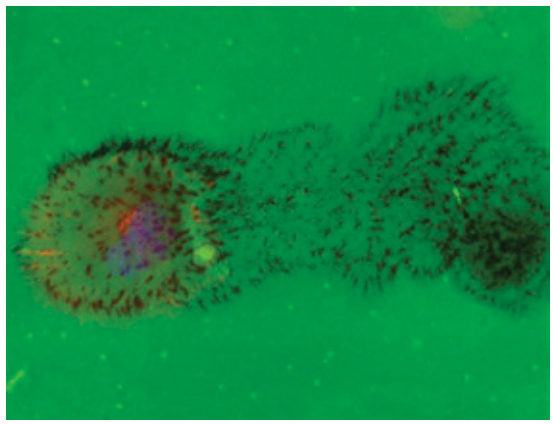
Tissue Invasion and Metastasis

Metastasis is the cumulative result of multiple changes in tumor cells and their microenvironment that enable cells to migrate to locations distant from the primary tumor and colonize the host tissue. Rampant angiogenesis facilitates metastasis, providing nutrients to the periphery of the primary tumor and easily penetrable vascular walls. Invasion of the stroma and vessel walls requires tumor cells to downregulate cell-cell adhesion (by altering expression of surface molecules and mimicking epithelial-mesenchymal transition (EMT)) and secrete matrix-degrading enzymes. After tumor cells survive circulation, they adhere to endothelial cells at the metastatic site, extravasate, and colonize the new host tissue. The ability to colonize distant sites is mediated both by tumor cell adhesion as well as premetastatic changes in the distant microenvironment that prepare for arriving metastatic cells. The mechanisms by which this "metastatic niche" is determined are still being studied.

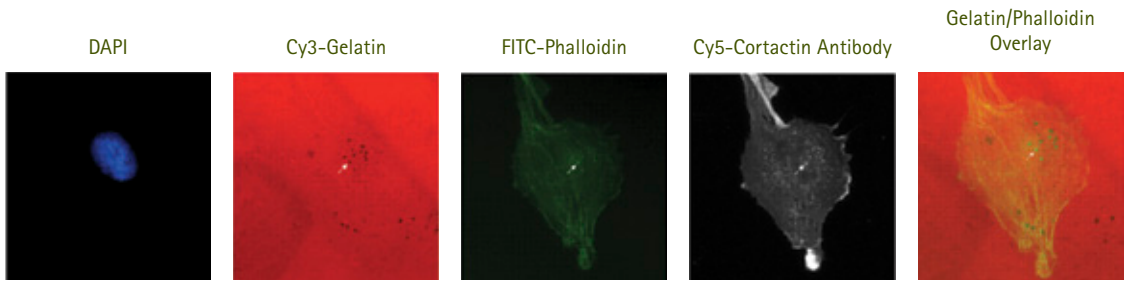
QCM™ Gelatin Invadopodia Assays (Green & Red)

(Catalogue Nos. ECM670 & ECM671)

Merck Millipore's QCM™ Gelatin Invadopodia Assays provide optimized materials and protocols to enable reproducible analysis of invadopodia in invasive tumor cells. All of the components necessary for affixing a thin film of fluorescent matrix to glass culture surfaces are provided. In addition, compatible reagents are provided for co-localizing the actin cytoskeleton and nuclei with invadopodial degradation sites. This assay may be used for assessing activity of inhibitors and promoters of invadopodia formation and function.



Cell migration across a fluorescently-conjugated matrix. Dark spots are evidence of invadopodia.



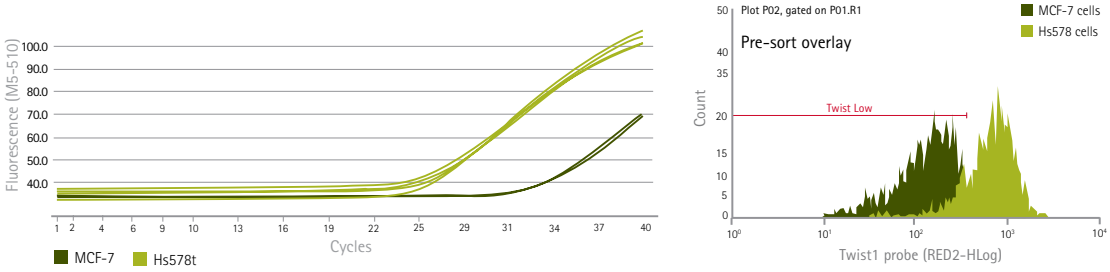
Co-localization of degradation with invadopodia-related puncta. RPMI-7951 human skin melanoma cells were seeded onto Cy3-conjugated gelatin substrates. Cells were fixed and incubated with a primary antibody against cortactin and Cy5-secondary antibody incubation was performed concurrently with FITC-Phalloidin and DAPI staining. Indicative of "active" invadopodia, white arrows in the gelatin, phalloidin, cortactin and overlay images demonstrate an example of co-localization between matrix degradation, F-actin puncta and cortactin foci (cortactin protein is strongly associated with actin assembly and invadopodia formation).

SmartFlare™ Twist Hu-Cy5 RNA Detection Reagent

(Catalogue No. SF-110)

Twist, a transcription factor that regulates embryonic development by selective induction of EMT in specific cells, also plays a key role in conferring metastatic potential on cancer cells. Using SmartFlare™ probes, which can reveal RNA levels in individual, live cells, is a powerful means of elucidating the relationship between Twist expression and the broader network of signaling involved in EMT, tumor cell invasion and metastasis.

Learn more about new SmartFlare™ technology at: **www.millipore.com/smartflare**



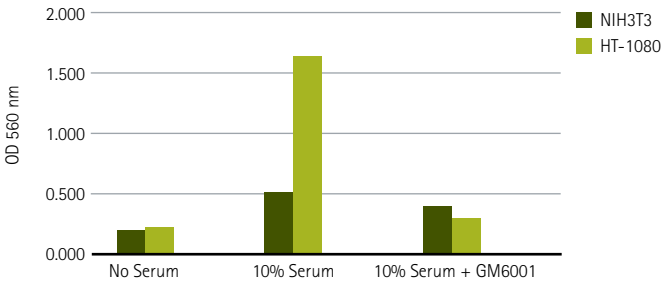
SmartFlare™ detection of Twist mRNA in individual cells provides richer information than qRT-PCR. As expected, Twist expression was determined by qRT-PCR to be higher in Hs578t cells when compared to MCF-7 cells (A). SmartFlare™ analysis of a 1:1 mixture of these two cell lines (B) showed the same trend; however, because we were able to analyze expression in individual, intact cells, we observed that MCF-7 cells exhibited a wider range of Twist expression than did the Hs578 cells, which produced a narrower, slightly bimodal peak. In contrast, the RT-PCR data merely provided the average level of gene expression present in the mixed lysate.

QCM™ Cell Invasion/Migration/Chemotaxis Assays

(Catalogue Nos. ECM551, ECM558, ECM510 ECM580 and others.

For a complete listing, see chart on the next page or visit: **www.merckmillpore.com/cancer**)

The most widely accepted *in vitro* approach to evaluating cell invasion as a function of metastasis is the Boyden chamber method. The porous membrane insert of a multiwell plate is layered with an extracellular matrix (ECM) or basement membrane protein to simulate the barriers invaded by tumor cells *in vivo*. Merck Millipore's QCM™ inclusive invasion, migration, and chemotaxis assays enable the investigation of various elements of cell movement during metastasis.



Matrix metalloprotease (MMP) inhibitor blocks invasion by HT-1080 cells. After applying a general MMP inhibitor (GM6001) to both a metastatic cancer cell line (HT-1080) and normal fibroblast cell line (NIH 3T3), cell invasion was initiated using 10% FBS as a chemoattractant and measured using the QCM™ Cell Invasion Assay (ECM551).

Cell Migration and Invasion Assay Table

Description	Pore Size	Plate Format	ECM Coating	Detection	No. of Tests	Catalogue No.
Chemotaxis Cell Migration Assays	8 µm	24-well	None	Colorimetric	24	ECM508
		24-well		Fluorometric	24	ECM509
		96-well		Fluorometric	96	ECM510
	5 µm	24-well		Colorimetric	24	ECM506
		24-well		Fluorometric	24	ECM507
		96-well		Fluorometric	96	ECM512
	3 µm	24-well		Colorimetric	24	ECM504
		24-well		Fluorometric	24	ECM505
		96-well		Fluorometric	96	ECM515
Haptotaxis Cell Migration Assays	8 µm	24-well	Fibronectin	Colorimetric	24	ECM580
		24-well	Vitronectin	Fluorometric	24	ECM581
		24-well	Collagen I	Fluorometric	24	ECM582
	5 µm	24-well	Laminin vials	Colorimetric	24	ECM220
		24-well		Fluorometric	24	ECM221
Millicell® µ-Migration Assay Kit	NA	4 slides of 3 wells		NA	12	MMA205
Cell Invasion Assays	8 µm	24-well	ECMatrix™	Colorimetric	12	ECM550
		24-well		Colorimetric	24	ECM554
		96-well		Colorimetric	96	ECM555
		24-well	Collagen I	Colorimetric	24	ECM551
		24-well		Colorimetric	24	ECM552
		96-well		Fluorometric	96	ECM556
High Sensitivity Cell Invasion Assay		24-well	Non-cross-linked Collagen	Colorimetric	24	ECM1401
Endothelial Cell Migration Assays	3 µm	24-well	Fibronectin	Colorimetric	24	ECM200
		24-well		Fluorometric	24	ECM201
Endothelial Cell Invasion Assays		24-well	ECMatrix™	Colorimetric	24	ECM210
		24-well		Fluorometric	24	ECM211
Leukocyte Transendothelial Migration	3 µm	24-well	Fibronectin	Colorimetric	24	ECM557
Tumor Cell Transendothelial Migration	8 µm	24-well		Colorimetric	24	ECM558
QCM™ Invadopodia Gelatin Degradation Assay (Green)	NA	NA	FITC-Gelatin*	Fluorometric	32	ECM670
QCM™ Invadopodia Gelatin Degradation Assay (Red)			Cy3-Gelatin*	Fluorometric	32	ECM671

*FITC-Gelatin and Cy3-Gelatin are provided but not pre-coated.

What pore size should I select?

Pore size determination depends entirely on your cell type. A quick literature search will enable you to decide the best pore size for the particular cells you are using. The following chart illustrates pore size choices for example cell lines used in our assays, and by some of our customers for these assays.

Cell Name	Cell Type	Pore Sizes	Assays typically performed
MDA-MB-231	Invasive breast cancer cell line (human)	5 or 8 µm	5 or 8 µm used in chemotaxis or invasion assay
MCF7	Non-invasive breast cancer cell line (human)	5 or 8 µm	5 or 8 µm used in chemotaxis or invasion assay
HT1080	Invasive fibrosarcoma cell line (human)	5 or 8 µm	5 or 8 µm used in chemotaxis or invasion assay
NIH3T3	Non-invasive fibroblast cell line (mouse)	5 or 8 µm	5 or 8 µm used in chemotaxis or invasion assay
HUVEC (Human vein umbilical vein endothelial cells)	Endothelial cells	3 or 5 or 8 µm	3 or 5 or 8 µm in chemotaxis, invasion, angiogenesis or transendothelial migration assays
HMVEC/HMEC(Human dermal microvascular	Endothelial cells	5 or 8 µm	5 or 8 µm in chemotaxis, invasion, angiogenesis or transendothelial migration assays
PMN	Polymorphonuclear neutrophils	1 or 3 µm	1 or 3 µm in chemotaxis assays
	Primary stromal cells	8 µm	No information available
	Epithelial cells	3 or 5 µm	No information available
	Human coronary artery smooth muscle cells	5 µm	No information available
Hepatic stellate cells	Myofibroblast	5 µm	No information available

MILLIPLEX® MAP Human MMP Panels 1 and 2

(Catalogue Nos. HMMP1-55K, HMMP2-55K)

The MMPs and tissue inhibitors of metalloproteases (TIMPs) are responsible for the balance in the breakdown of extracellular matrix (ECM), playing a key role in normal physiological processes, such as embryonic development and tissue morphogenesis. Disruption of the MMP/TIMP balance can result tumor growth and metastasis. The MILLIPLEX® MAP Human MMP and TIMP Panels enable you to explore the modulation of TIMP expression and the function of the MMP/TIMP balance.

Use our easy online tool to design your MILLIPLEX® MAP kits. Simply choose the species, panel, and analytes that best fit your needs, and Merck Millipore will build the kit to your specifications.For more information, visit: **www.millipore.com/kits**

Cell Comb™ Scratch Assay

(Catalogue No. 17-10191)

Cell migration can be studied by a variety of methods, but the scratch assay remains a highly popular method due to the simplicity of the required materials, experimental setup, data collection and interpretation. Merck Millipore has developed the Cell Comb™ Scratch Assay to address the need for an easy-to-use tool for creating multiple scratch wounds. The patent pending Cell Comb™ has been optimized to apply a high density field of scratches to maximize the area of wound edges, while leaving sufficient numbers of undamaged cells to migrate into the gap. This form of high density wounding creates a high proportion of migrating cells to quiescent monolayer cells, which permits sensitive detection of the biochemical events occurring, specifically in the migrating cell population.

Key Products for Metastasis

Antibodies and Proteins

Description	Catalogue No.
Anti-Integrin aVb3, clone LM609, azide free	MAB1976Z
Anti-MMP-9, catalytic domain	AB19016
Anti-Met (extracellular), clone 4F8.2	05-1049
Met (D1246N), active	14-818
TIMP-2, human, recombinant	PF021

For our entire line of cell migration, invasion, metastasis, cell adhesion, and extracellular matrix antibodies for cancer, search: **www.millipore.com/antibodies**

Assays

Description	Catalogue No.
Endothelial Cell Adhesion Assay	ECM645
Integrin-mediated & ECM Cell Adhesion Arrays	ECM530 and others
Quantitative Pseudopodia Assay Kit	ECM650
Ready-to-Assay™ LPA1 Lysophospholipid Receptor Frozen Cells	HTS089RTA
ChemiScreen™ EP2 Prostanoid Receptor Membrane Preps	HTS185M
ChemiScreen™ LPA1 Lysophospholipid Membrane Preps	HTS089M

Inhibitors

Description	Catalogue No.
GM6001 (MMP inhibitor)	364206
MMP Inhibitor Set I	444255
TGFb RI Inhibitor II	616452

For a complete listing of cancer-related products from Merck Millipore, please visit: **www.merckmillipore.com/cancer**

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Hallmarks of Cancer


Self-sufficiency in proliferation signals

Insensitivity to proliferation inhibiting signals


Evading apoptosis


Limitless replicative potential


Sustained angiogenesis

Metabolic reprogramming


Escape from immune control


Genome instability and mutation


Tumor-promoting inflammation


Tissue invasion and metastasis

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