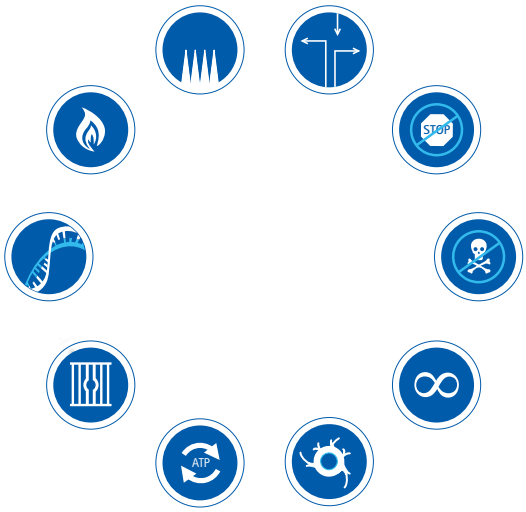
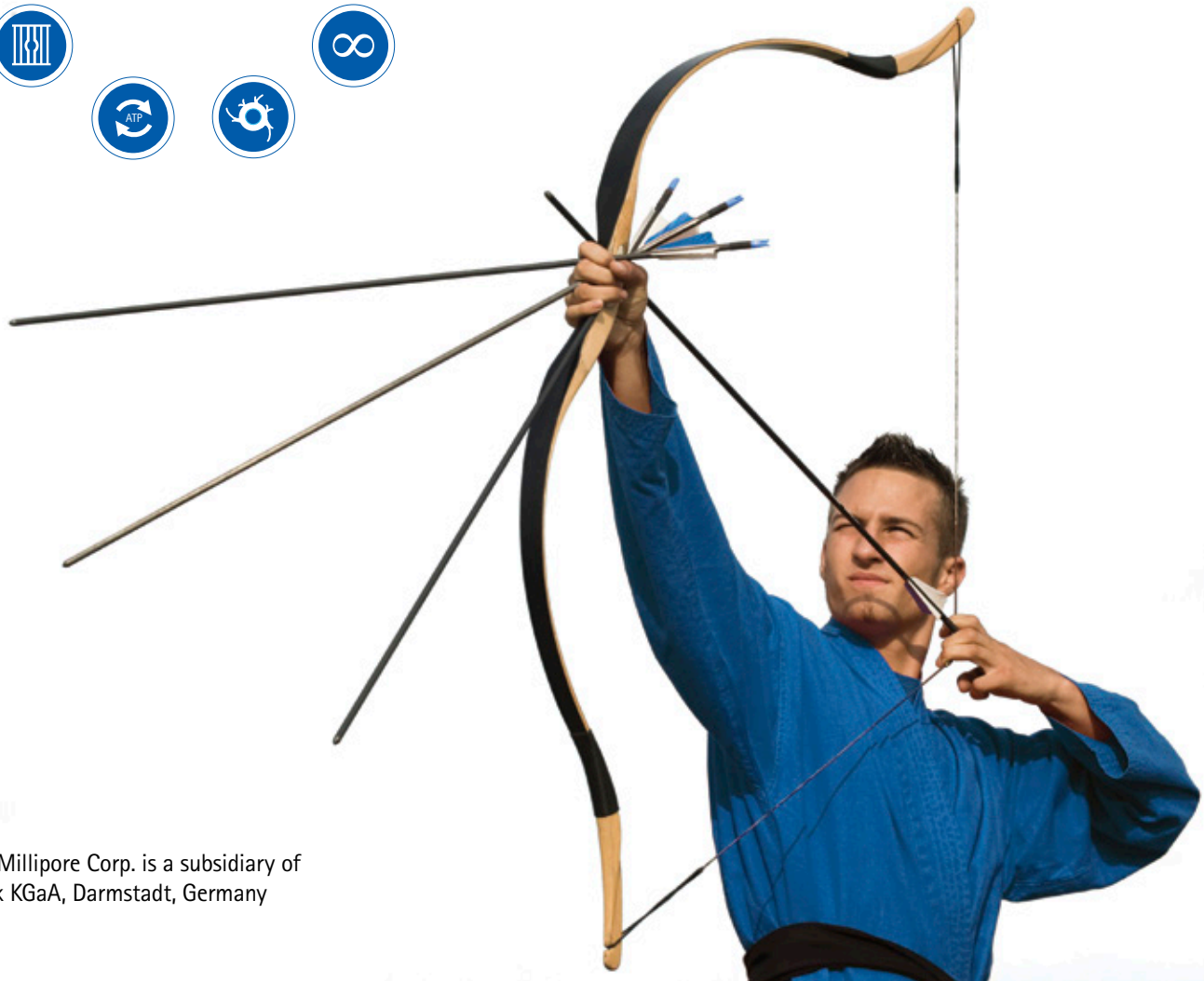
Hallmarks of Cancer

Solutions for life science research





EMD Millipore Corp. is a subsidiary of
Merck KGaA, Darmstadt, Germany

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 between tumor and non-tumor cells has yet to be fully mined, the data point to ten key traits (listed in the table of contents), shared by most tumors as the ones that drive disease progression. These "hallmarks" of cancer, as described by Hanahan and Weinberg*, are important—not only because they represent opportunities for therapeutic intervention, but also because they provide 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 the 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 intrinsic from extrinsic factors require an unbiased, multidisciplinary, multiparametric, cross-platform approach. Recognizing the opportunities and challenges facing cancer research, EMD Millipore has been dedicated to developing and refining tools and technologies for the study of cancer. With EMD 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.

In this guide, you will find key concepts and latest findings related to the hallmarks of cancer, and also discover EMD Millipore's solutions for the investigation of the processes associated with these hallmarks. With so many effective solutions, it is our hope that you will advance cancer-related life science research with EMD Millipore as your partner.

Sincerely,
EMD Millipore

*Hanahan D., Weinberg R.A. (2011) Hallmarks of Cancer: The Next Generation. Cell. 144(5):646-74.

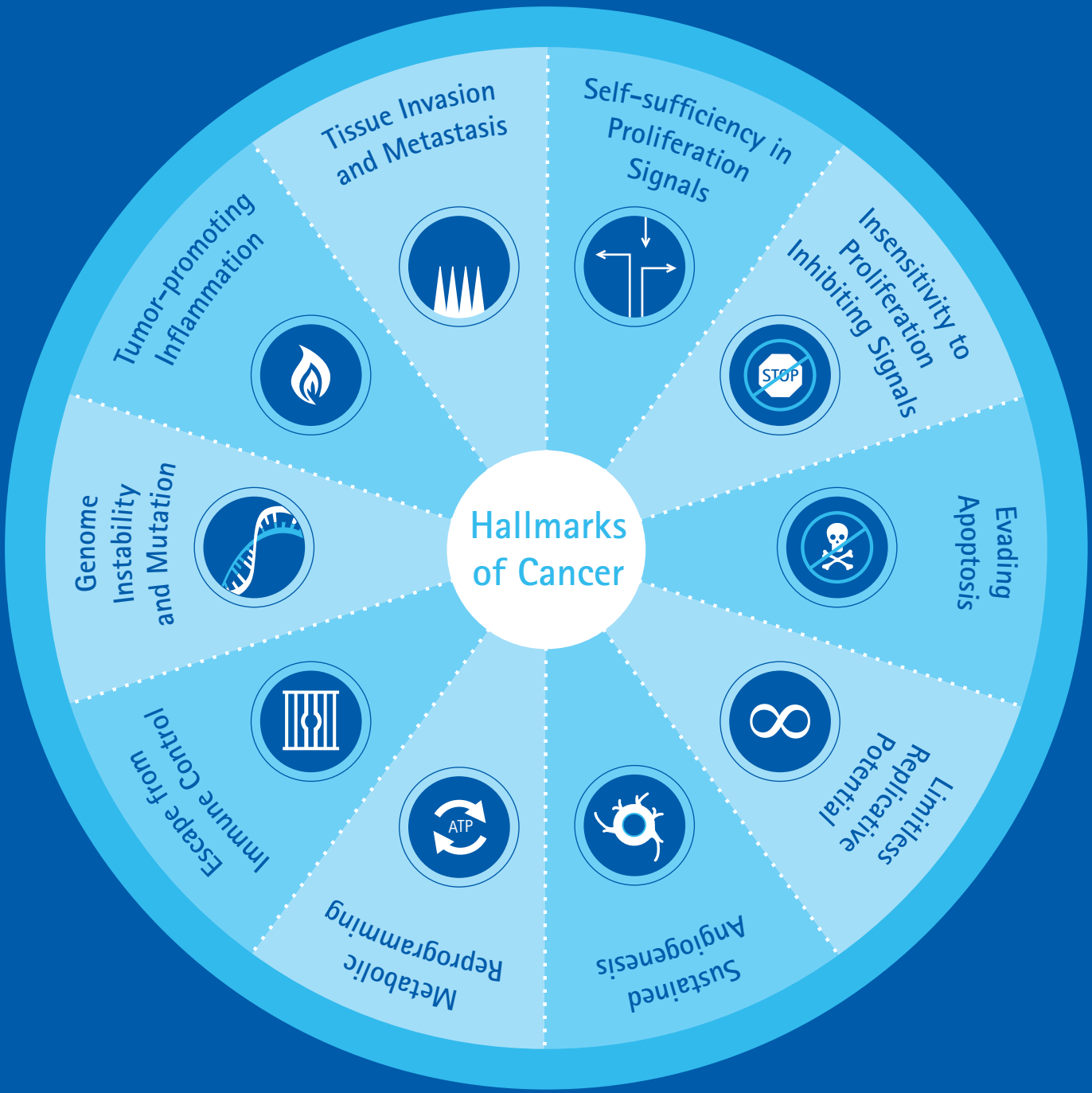

Hallmarks of Cancer
Self-sufficiency in Proliferation Signals
Insensitivity to Proliferation Inhibiting Signals
STOP
Evading Apoptosis
Limitless Replicative Potential
Sustained Angiogenesis
Metabolic Reprogramming
ATP
Escape from Immune Control
Genome Instability and Mutation
Tumor-promoting Inflammation
Tissue Invasion and Metastasis

Table of Contents

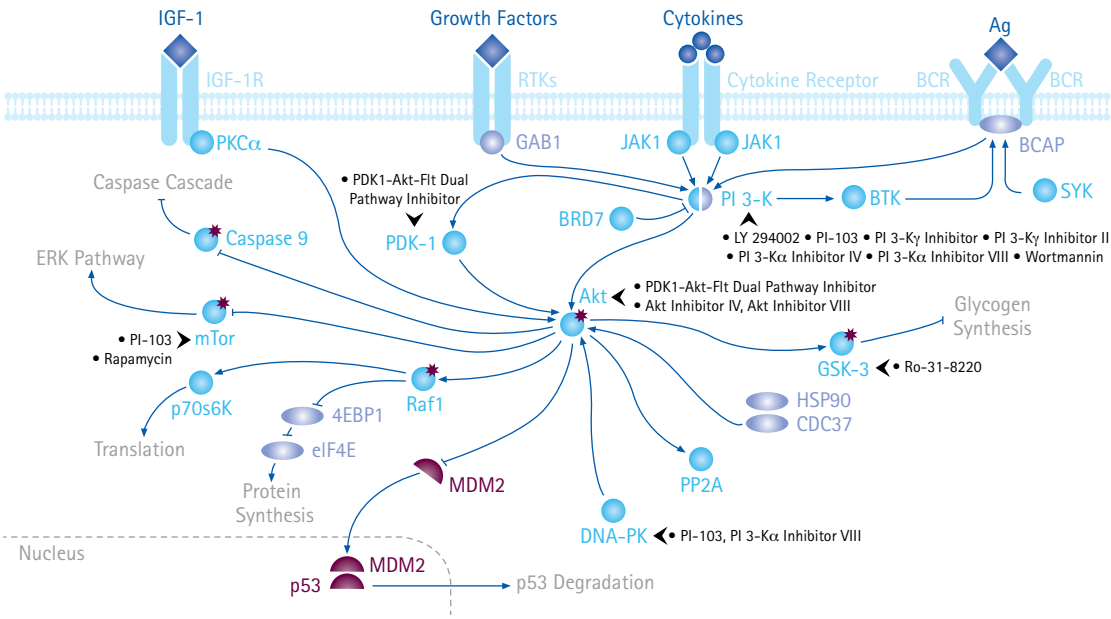
Self-sufficiency in proliferation signals

Unlike normal cells, cancer cells don't depend on extrinsic growth factors and cytokines to proliferate.

Dysregulated PI3-kinase/Akt signaling has been reported in a variety of human tumors. Over 30% of solid tumors are reported to contain mutations in a catalytic unit of PI3-kinase. These mutations increase its enzymatic activity above normal, stimulating Akt signaling and allowing growth factor-independent proliferation. Not only are the PI3-kinase/Akt pathway's inputs diverse (shown in the pathway below), but it also has numerous effector pathways, and sometimes multiple proteins in the network are simultaneously mutated in cancers.

Despite the massive efforts to develop therapeutic compounds to block PI3-kinase, the enzyme's multiple isoforms of its subunits, multiple functions in normal cells, and ostensibly redundant signaling effectors have made it difficult to find a sufficiently specific compound with manageable side effects.

As a result, research into the regulation of this pathway continues. Recent discoveries include identification of BRD7, a bromodomain-containing protein that negatively regulates PI3-kinase[1] and new strategies for selectively inhibiting Bruton's Tyrosine Kinase (BTK), which is both an effector and regulator of PI3-kinase[2]. The scope of the PI3-kinase/Akt signaling network has now broadened to include miRNA-mediated gene regulation and chromatin modification.



The PI3-kinase/Akt pathway integrates multiple signals, such as nutrients (sugars and amino acids), growth factors, cytokines and immune cell-activating antigens.

Fortunately, the development of research-use antibodies and small molecule inhibitors targeted to multiple proteins up and down this pathway, many from EMD Millipore, has paved the way to elucidating the dynamics of this complex signaling network.

Featured Publications

1. Chiu YH, et al. BRD7, a tumor suppressor, interacts with p85α and regulates PI3K activity. Mol Cell. 2014 Apr 10;54(1):193-202.
2. Davids MS, Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. Future Oncol. 2014 May;10(6):957-67.
3. Bertacchini J, et al. Feedbacks and adaptive capabilities of the PI3K/Akt/mTOR axis in acute myeloid leukemia revealed by pathway selective inhibition and phosphoproteome analysis. Leukemia. 2014 Nov;28(11):2197-205.

Proliferation Signaling

Featured Technique:

Cell Proliferation Assays

Evaluation of cell proliferation is essential for studies of most biological processes and for many cell based assays. The traditional method for detection of cell proliferation has been the measurement of [^{3}H]-thymidine incorporation as cells enter S phase, and subsequent quantification of [^{3}H]-thymidine, as performed by scintillation counting. This technology is slow, labor-intensive and has several limitations, including the handling and disposal of radioisotopes and the necessity of expensive equipment. EMD Millipore has developed multiple technologies for biomolecular detection and cellular analysis that offer significant advantages over [^{3}H]-thymidine incorporation for quantifying cell proliferation with speed, precision, and accuracy. These include the use of non-radioactive reagents such as EdU, BrdU, WST-1, and MTT.

Featured Solution:

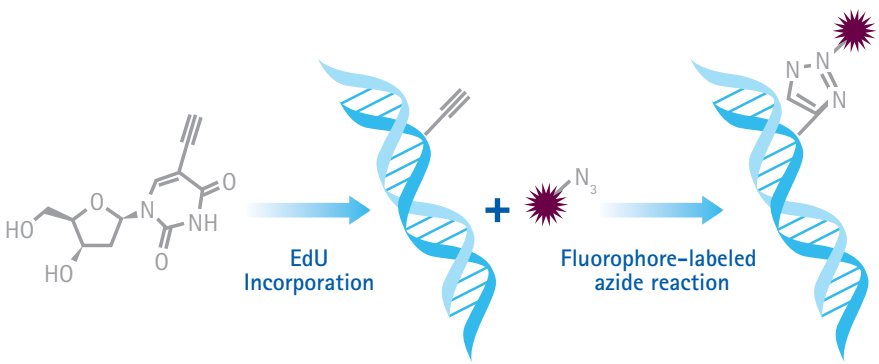
NEW EdU Cell Proliferation Assays

(Catalog Nos. 17-10525, 17-10526, 17-10527, 17-10528)

The use of EdU (5-ethynyl-2'-deoxyuridine) as a thymidine nucleoside analog is a significant improvement compared to the classical BrdU and [^{3}H]-thymidine cell proliferation assays. In contrast to BrdU assay kits, the EdU cell proliferation assays are not antibody-based and do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the EdU Cell Proliferation assays measure the incorporation of EdU into newly synthesized DNA with click chemistry for detection by fluorescence microscopy and flow cytometry.

Features and Benefits

- Fast, simple workflow
- No DNA denaturation step
- No antibody
- Structurally preserves sample
- Fast detection procedure (~30 minutes)
- Compatible with flow cytometry and fluorescence microscopy
- Kits available with a variety of high resolution fluorescent readouts (EdU488, 555, 594, 647)



EdU Cell Proliferation Assay Principle. Cells grown in the presence of 5-EdU incorporate the compound at thymidine bases during S-phase. Fluorophore-labeled azide reacts with the incorporated EdU to allow detection by microscopy or flow cytometry.

Technical Tip

When studying Akt1, make sure to measure both Ser473 and Thr308 phospho sites, as both are required for full Akt activation.

The Hallmark & Drug Development

Ibrutinib (trade name IMBRUVICA®) modulates the PI3-kinase signaling in B cells by covalently inhibiting the BTK kinase. It has been approved for treating mantle cell lymphoma and chronic lymphocytic leukemia (CLL).

Featured Cancer: Head and Neck Squamous Cell Carcinoma

Historically, many patients with this cancer type were being treated with EGFR modulators, but eventually developed resistance to these drugs. Recently, some of these patients responded to alpelisib, an inhibitor specific to the PI3-Kα.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Proliferation Signaling:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-phospho-Akt (Thr308), clone 50-1C-25, rabbit monoclonal	05-802R
	Anti-phospho-erbB-2/HER-2 (Tyr1248)	06-229
	Anti-IRS-2, clone 9.5.2	MABS15
	Anti-phospho-IRS-2 (Ser388)	07-1517
	Anti-mTOR, clone 2ID8.2	05-1592
	Anti-APC	MABC202
	Anti-SHP-1, clone EPR5519, Rabbit Monoclonal	MABC773
	Anti-USP18 (UBP43), clone 1C3.1	MABC113
	Anti-Cyclin A2, clone 17B8.2	MABC205
	Anti-ANGPTL6	ABC84
	PI3 Kinase/Akt/GSK3 Pathway Explorer Minipack	15-103
	Anti-PI3 kinase α, clone 2B12.1	MABS76
	Anti-PI3 Kinase β, p110b, clone 8C3.2	05-1558
	Anti-PI3 Kinase, p85 Protein, agarose conjugate, 200 µg	16-107
	Anti-PI3 Kinase, p110δ rabbit monoclonal	04-401
	Anti-Akt, PH Domain, clone SKB1	ST1088
	Anti-Akt1/PKBα	07-416
	Anti-Akt/PKB, PH Domain, clone SKB1, Magnetic Bead Conjugate	16-318
	Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 488	16-293
	Anti-Akt2/PKBβ, clone AW114, rabbit monoclonal	05-771
	Anti-Akt/PKB, PH Domain, clone SKB1	05-591MG
	Milli-Mark® Anti-Akt/PKB-Alexa Fluor® 647, clone SKB1	FCMAB128A6
	Anti-phospho-AKT (Thr34)	07-789
	Anti-phospho-Akt (Thr308)	07-1398
	Anti-phospho-Akt1 (Tyr326)	09-288
	Anti-phospho-Akt (Ser473), clone 6F5	05-1003MG
	Anti-mTOR, rabbit monoclonal	04-385
	Anti-mTOR, clone 21A12.2	05-1564MG
	Anti-mTOR/FRAP, clone 22C2	MABS196
	Anti-mTOR, clone F11	MABS14
	Anti-mTOR, clone 2ID8.2	05-1592
	Anti-phospho-mTOR (Ser2448)	09-213
	Anti-phospho-mTOR (Ser2481)	09-343
	Anti-phospho-mTOR (Ser2159)	ABS79

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Proliferation Signaling (continued):

Research Solutions	Description	Catalog No.
Related Antibodies (continued)	Anti-phospho-mTOR (Thr2164)	ABS88
	Anti-Raptor	09-217
	Anti-phospho-Raptor (Ser722)	09-104
	Anti-Ras, (K-, H-, N-), clone 9A11.2	05-1072
Related Proteins	EGFR, active, purified kinase	14-531
	Thrombopoietin	GF037
	Amphiregulin, Human, Recombinant	171372
Cell Proliferation Assays	EdU Cell Proliferation Assay (EdU-488)	17-10525
	EdU Cell Proliferation Assay (EdU-555)	17-10526
	EdU Cell Proliferation Assay (EdU-594)	17-10527
	EdU Cell Proliferation Assay (EdU-647)	17-10528
	Cell Proliferation Assay Kit, WST dye	2210
	BrdU Cell Proliferation Kit (200 assays)	2750
	BrdU Cell Proliferation Kit (1000 assays)	2752
	BrdU Cell Proliferation Assay	QIA58
Growth and Viability Assays	MTT Cell Growth Assay Kit (5000 assays)	CT01
	MTT Cell Growth Assay Kit (1000 assays)	CT02
	Cellular Senescence Assay	KAA002
	Muse® Count & Viability Assay Kit	MCH100102
	Guava® Cell Growth Kit for Flow Cytometry	4500-0270
Kinase Assays	PI3-Kinase Activity/Inhibitor ELISA	17-493
	PI3-Kinase HTRF® Screening Assay	33-016
	Raf-1 Kinase Assay Kit, Chemiluminescence Detection	17-360
	Ras Activation Assay Kit	17-218
	Rac1 Activation Assay Kit	17-283
	Rho Activation Assay Kit	17-294
	Rac1/Cdc42 Activation Assay Kit	17-441
	Rac1/cdc42 Activation Magnetic Beads Pulldown Assay	17-10394
	PIP_3 Quantification TR-FRET Assay	17-494
	c-ErbB2/c-Neu Rapid Format ELISA Kit	QIA10
	K-LISA™ mTOR (Recombinant) Activity Kit	CBA104
	Phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) STAR ELISA Assay Kit	17-464
	FlowCellect® MAPK Activation Dual Detection kit	FCCS025106
	FlowCellect® PI3-K Activation Dual Detection Kit	FCCS025105
	FlowCellect® EGFR/MAPK Pathway Activation Detection Kit	FCCS025101
	FlowCellect® PI3-K/MAPK Dual Pathway Activation and Cancer Marker Detection Kit	FCCS025100

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Proliferation Signaling (continued):

Research Solutions	Description	Catalog No.
Multiplex Analysis Kits	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
Live Cell RNA Detection	SmartFlare™ EGFR Hu-Cy5 RNA Detection Reagent	SF-151
Compound Libraries	InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I	539744
	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
Small Molecules	Cell Proliferation Inducer, WS3	506028
	Akt Inhibitor	124005
	Akt Inhibitor II	124008
	Akt Inhibitor III	124009
	Akt Inhibitor IV	124011
	Akt Inhibitor V, Triciribine	124012
	Akt Inhibitor VI, Akt-in	124013
	Akt Inhibitor VII, TAT-Akt-in	124014
	Akt Inhibitor VIII, Isozyme-Selective	124018
	Akt Inhibitor XV, Isozyme-Selective	124034
	Akt Inhibitor XVIII, SC66	124036
	Akt Inhibitor XIX, 3CAI	124037
	PDK1/Akt/Flt Dual Pathway Inhibitor *KP-	521275
	InhibitorSelectAkt/PI 3-K/mTORSignalingP	124031
	LY294002	440202
	PI103	528100
	Wortmannin	681675
	mTOR Kinase Inhibitor II, WYE-354	475986
	mTOR Inhibitor III, PP242	475988
	mTOR Inhibitor IV, Ku-63794	475990
	PI 3-K/mTOR Inhibitor III, PKI-179	526561
	PI3Kd inhibitor, SW30	526559
	mTOR Inhibitor XI, Torin1	475991
	PTK/PI 3-K/mTOR Inhibitor, PP121	529584
	mTOR Inhibitor XII, Torin2	475992
	InhibitorSelect™ mTOR Signaling Regulators Panel	475995
	mTOR Inhibitor XI, ETP-46464	500508
	Lenaldekar (LDK)	531068

For a complete selection, visit: www.emdmillipore.com

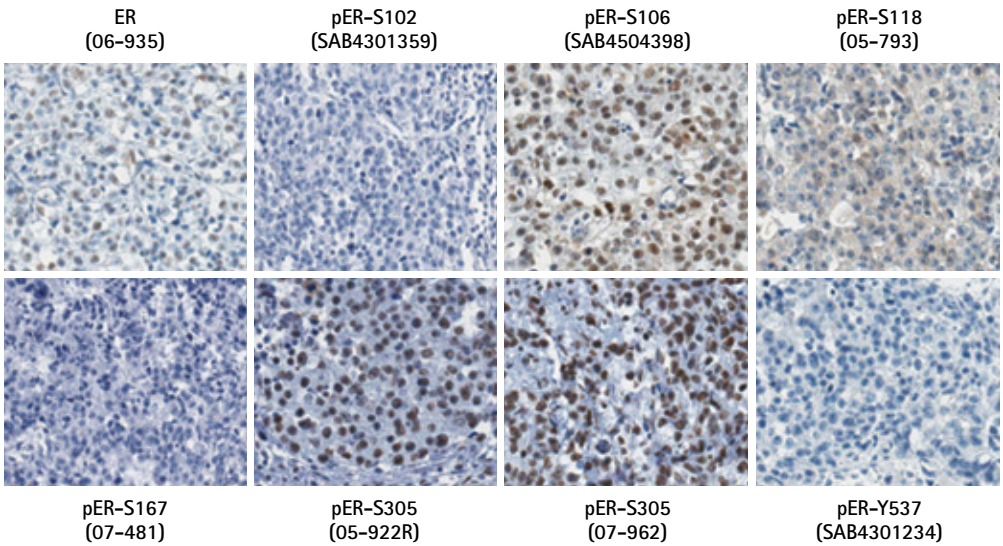
TECHNOLOGY HIGHLIGHT

Immunohistochemistry of Phosphorylated ERα in Breast Cancer Tissue using the SNAP i.d.® system for IHC

Estrogen receptor α (ERα) is phosphorylated on multiple residues when activated by ligands and growth factors. In tumors, phosphorylation may be present even in the absence of these stimuli, suggesting that measuring ERα phosphorylation may be useful for identifying breast cancer tissue.

We employed antibodies that specifically recognized certain phosphorylated sites on the ERα nuclear receptor and validated them for immunohistochemistry, recognizing that most breast cancer researchers would need to identify tumor cells within heterogeneous tissue sections.

In order to perform multiple IHC reactions in parallel, the SNAP i.d.® medium-throughput IHC system was used. We were able to stain up to 24 slides at a time with vacuum-driven removal of staining solutions from slides, streamlining the IHC process and promoting reproducibility.



Example of breast cancer tissue sections analyzed by IHC, using indicated antibodies and the SNAP i.d.® 2.0 system for IHC.

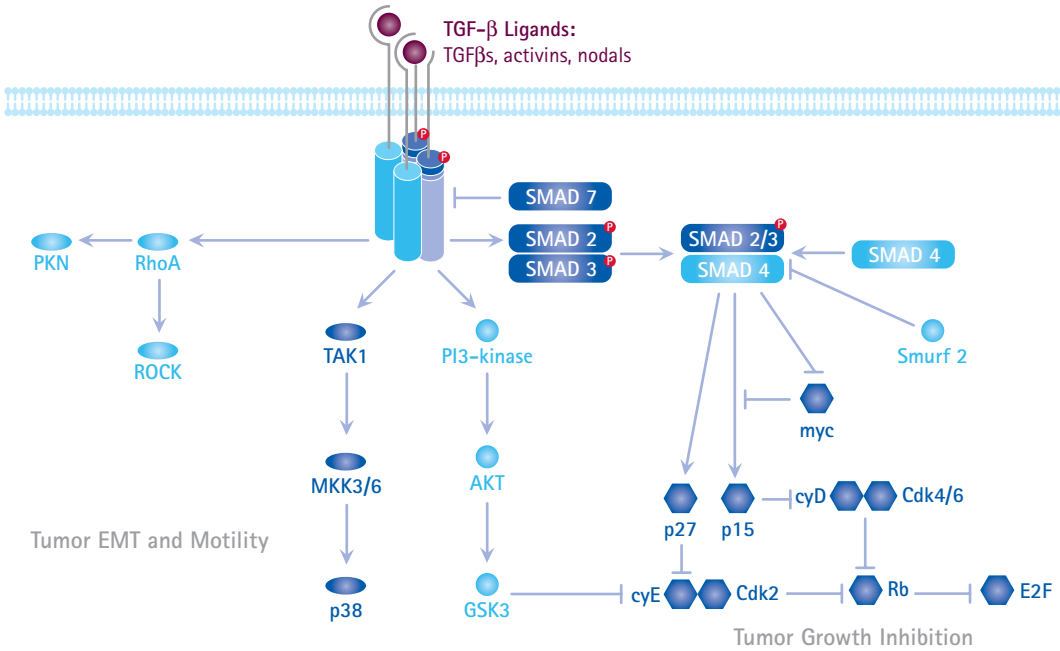
Insensitivity to proliferation-inhibiting signals

Sometimes "stop" signs double as "go" signs: activin, Nodal, Lefty and other TGFβs.

Transforming growth factor β (TGFβ) family proteins suppress cell proliferation by binding to TGFβ receptors, which transduce signals that arrest the cell cycle in the G1 phase. In addition to the well-described, canonical, SMAD-mediated effect of TGFβ signaling on cell cycle and transcription, many noncanonical pathways, including those mediated by Ras, Akt, RhoA, NF-κB, Hedgehog, and Notch, also are part of this complex network. And SMADs, it turns out, not only act as transcription factors, but also mediate miRNA processing via Drosha.

To further complicate matters, TGFβ signaling may also induce epithelial-to-mesenchymal transition and suppress immune surveillance, thereby promote tumor progression. Perhaps this is why tumor cells actually show upregulation of TGFβ, to a point at which its tumor promoting function overwhelms its tumor suppressive function. The molecular details behind this "switch" are only now being fully understood[1].

Many drug candidates are being developed to block TGFβ signaling at various points, which include receptor phosphorylation, ligand activation, and ligand binding.



The TGFβ signaling network inhibits tumor cell proliferation but also promotes tumor cell motility, inflammation, and suppresses immune cell proliferation.

Featured Publications

1. Xu J et al. 14-3-3ζ turns TGF-β's function from tumor suppressor to metastasis promoter in breast cancer by contextual changes of Smad partners from p53 to Gli2. Cancer Cell. 2015 Feb 9;27(2):177-92.
2. Yuan JH, et al. A long noncoding RNA activated by TGF-β promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell. 2014 May 12;25(5):666-81.
3. Togashi Y, et al. Activin signal promotes cancer progression and is involved in cachexia in a subset of pancreatic cancer. Cancer Lett. 2015 Jan 28;356(2 Pt B):819-27.

Proliferation Inhibition

Featured Technique:

Chemical Genetics

Chemical genetics has been used successfully as both gain-of-function and loss-of-function approaches to study proliferation inhibition signaling. For example, in chemical genetics, either small organic molecules or peptides are used to activate or inhibit specific proteins/enzymes involved in specific signal transduction pathways. Chemical genetics is technically simple to perform in cell culture, and requires fewer resources and less time. Small molecules provide the advantage of acting quickly, and the effects can often be reversed rapidly by simply washing. The fast action of most small molecules also makes them ideal for live imaging. In addition, the use of small molecules can provide dose response information.

Small molecules offer a powerful approach to temporally and spatially modulate individual proteins and processes that can control biological phenotypes. They have helped to identify signaling molecules that define and maintain the extensive intracellular communication networks that control growth, differentiation, metabolism, and other critical cellular functions.

Reference:
1. Eggert, U.S. et al. (2006) Small molecules in an RNAi world. Mol.BioSyst. 2, 93-96.

Featured Solution:

InhibitorSelect™ 96-Well Protein Tyrosine Kinase & Protein Tyrosine Phosphatase Inhibitor Library IV

(Catalog No. 539747)

Recognizing both the tremendous opportunities and critical role of protein kinases, we have introduced several Calbiochem® InhibitorSelect™ Libraries that contain collections of carefully selected, structurally diverse, and potent small molecules targeting members from several families of protein kinases in convenient, cost effective formats.

Well-characterized: Unlike small molecule libraries from other leading suppliers, InhibitorSelect™ libraries are provided with documented cell permeability, reversibility, potency, published IC_{50}/K_i values, lot-specific data, stability, and HPLC purity for most of the included molecules.
Well-documented: Comprehensive documentation about each inhibitor, such as molecular structure, IC_{50} values, literature citations, and CAS number (where available), is provided at your fingertips.

The InhibitorSelect™ 96-Well Protein Tyrosine Kinase & Protein Tyrosine Phosphatase Inhibitor Library IV consists of 83 pharmacologically active, potent protein kinase and phosphatase inhibitors: most of the inhibitors included in this library are cell-permeable and reversible in their action. A majority of tyrosine kinase inhibitors included act in an ATP-competitive manner. The inhibitors in this library will be useful for target identification in drug discovery, biochemical pathway analysis, screening of new protein kinases/phosphatases, and other pharmaceutical-related applications.

Enzyme Family	Number of Inhibitors in Library
Receptor Tyrosine Kinase (RTK)	37
Tyrosine Kinase (TK)	27
Tyrosine Kinase-like (TKL)	6
PKA, PKG, PKC families (AGC)	1
CDK, MAPK, GSK-3, CLK families (CMGC)	3
Receptor Tyrosine Phosphatase (RTP)	1
Tyrosine Phosphatase (TP)	8

This InhibitorSelect™ panel contains inhibitors targeting multiple kinase and phosphatase families.

Technical Tip

If you are studying protein:protein interactions using co-immunoprecipitation Western blot (coIP), and the coeluted capture antibody's heavy or light chain interferes with target protein detection, try cross-linking IP (covalently linking capture antibody to Protein A/G) to avoid antibody coelution.

The Hallmark & Drug Development

The TGFβ receptor I kinase inhibitor, galunisertib, is currently in phase II clinical trials for the treatment of glioma, hepatocellular carcinoma, and pancreatic cancer.

Featured Cancer

Glioblastoma, a type of brain cancer, typically has highly elevated TGFβ signaling. By either blocking the production or secretion of TGFβ proteins, certain glioblastoma drug candidates in late phase clinical trials aim to inhibit tumor cell invasion and proliferation as well as suppress immune evasion.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Proliferation Inhibition:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-Akt/PKB, PH Domain, clone SKB1, magnetic bead conjugate	16-318
	Anti-APC, clone CC-1	MABC200
	Anti-Cdk2	07-631
	Anti-Cdk2, clone AN4.3	05-596
	Anti-Cdk4	07-659
	Anti-Cdk4, clone DCS-35	MAB8879
	Anti-Cdk4, clone EPR4513, rabbit monoclonal	MABE548
	Anti-Cdk5, clone DC17	05-364
	Anti-Cdk6	ABC275
	Anti-Cdk6, clone 1F9.1	MABC280
	Anti-CLEC2A	ABC328
	Anti-CLEC2A, clone 8D2.1	MABC664
	Anti-Cyclin B1, clone GNS3 (8A5D12)	05-373
	Anti-LAP (TGF-β1) (human), FITC, clone TW4-2F8	MABF347
	Anti-LDH-A, clone 5D2.1	MABC150
	Anti-p38/SAPK2A	ABS29
	Anti-p38γ/SAPK3	07-474
	Anti-phospho-p38 (Thr180/Tyr182), clone 6E5.2	MABS64
	Anti-phospho-p38α (Thr180/Tyr182), clone 8.78.8, rabbit monoclonal	05-1059
	Anti-phospho-SMAD3 (Tyr179)	ABS47
	Anti-Smad2/3 Antibody	07-408
	Anti-Smad3, clone EP568Y, rabbit monoclonal	04-1035
	Anti-Smad4 (CT), clone EP618Y, rabbit monoclonal	04-1033
	Anti-SMAD6, clone 5H3	MABC683
	Anti-Smad7, clone EPR622, rabbit monoclonal	MABD187
	Cell Cycle-G2/M Phase Pathway Explorer Antibody Minipack	15-120
Related Proteins	TGFBR-1, active	14-912
Assays	FlowCellect® Bivariate Cell Cycle Kit for DNA Replication	FCCH025102
	FlowCellect® Bivariate Cell Cycle Kit for G2/M Analysis	FCCH025103
	EdU Cell Proliferation Assay (EdU-488)	17-10525
	EdU Cell Proliferation Assay (EdU-555)	17-10526
	EdU Cell Proliferation Assay (EdU-594)	17-10527
	EdU Cell Proliferation Assay (EdU-647)	17-10528
	Cell Proliferation Assay Kit, WST dye	2210
	BrdU Cell Proliferation Kit (200 assays)	2750
	BrdU Cell Proliferation Kit (1000 assays)	2752
	BrdU Cell Proliferation Assay	QIA58

For a complete solution, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Proliferation Inhibition:

Research Solutions	Description	Catalog No.
Assays (continued)	Guava® Cell Cycle Reagent Propidium Iodide Solution	4500-0220
	Muse® Cell Cycle Kit	MCH100106
	Phopsho-Akt (Thr473) STAR ELISA Kit	17-457
Multiplex Analysis Kits	MILLIPLEX® MAP Multi-species TGFβ 3-Plex	TGFB-64K-03
	MILLIPLEX® MAP TGFβ Signaling Pathway 6-Plex	48-614MAG
	MILLIPLEX® MAP Human Src Family Kinase Active Site Magnetic Bead Multiplex Assay – 8-Plex	48-650MAG
	MILLIPLEX® MAP MAPmate™ Assay, retinoblastoma (Rb) Thr252	46-704
Live Cell RNA Detection	SmartFlare™ miR-155-5p Hu-Cy3	SF-183
Compound Libraries	InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I	539744
	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
Small Molecules	TGF-β RI Kinase Inhibitor II	616452
	Chk2 Inhibitor II	220486
	Cdk2 Inhibitor III	238803
	Alsterpaullone	126870
	Cdk2 Inhibitor IV, NU6140	238804
	Cdk2/9 Inhibitor	238806
	Cdk4 Inhibitor	219476
	Cdk4 Inhibitor II, NSC 625987	219477
	Cdk4 Inhibitor III	219478
	Cdk4 Inhibitor V	219503
	Cdk4/6 Inhibitor IV	219492
	Olomoucine	495620
	p38 MAP Kinase Inhibitor	506126
	InSolution™ p38 MAPK Inhibitor III	506148
	p38 MAP Kinase Inhibitor IV	506153
	p38 MAP Kinase Inhibitor V	506156
	p38 MAP Kinase Inhibitor VI, JX401	506157
	p38 MAP Kinase Inhibitor VII, SD-169	506158
	Roscovitine	557360
	Smad3 Inhibitor, SIS3	566405
	Wee1 Inhibitor	681640
	SB 218078	559402
	TGF-β R1 Kinase Inhibitor III	616453
	Cdk1 Inhibitor IV, RO-3306	217699

For a complete solution, visit: www.emdmillipore.com

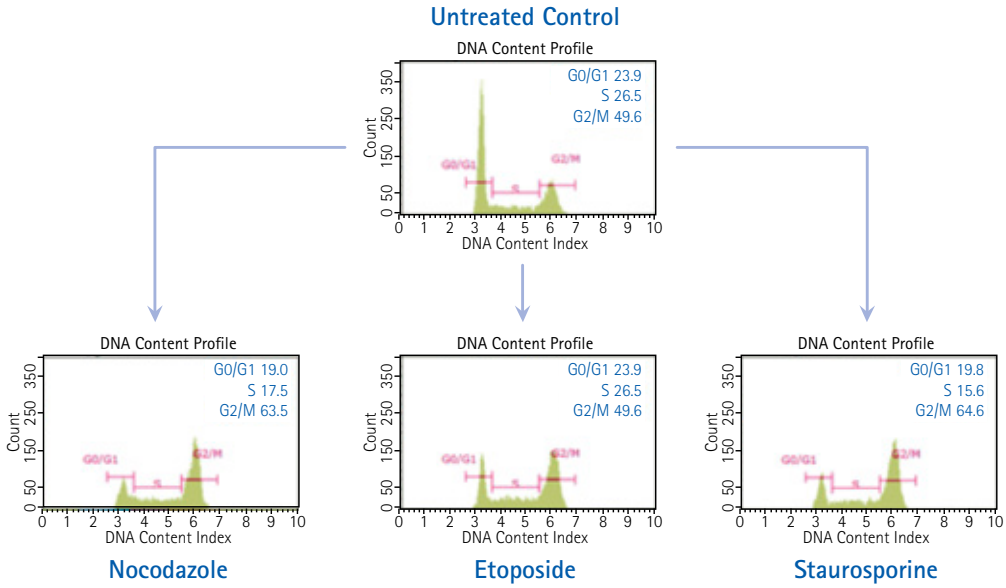
TECHNOLOGY HIGHLIGHT

Measuring cell cycle progression in single cells using the ultracompact Muse® cell analyzer

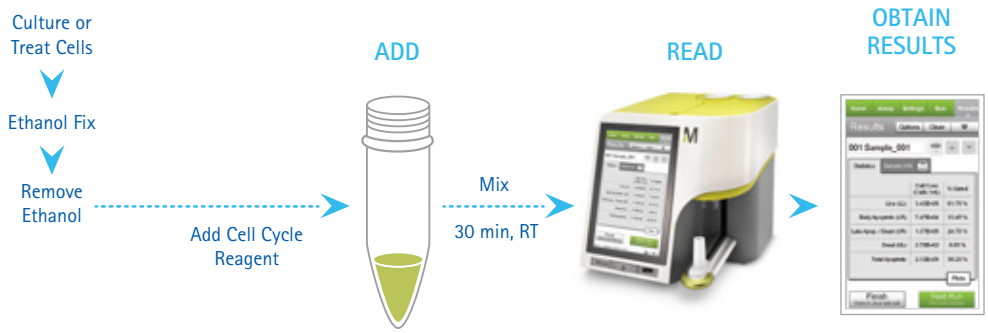
Flow cytometry is the most trusted method for determining the cell cycle distribution of a cell population. Particularly when analyzing the dynamics of a complex signaling network, it is vital to know the cell cycle distribution at the exact time of network analysis. In such cases, having an ultracompact flow cytometer like the Muse® cell analyzer can save a researcher a trip across the hall or across the campus.

The Muse® Cell Cycle Assay (Catalog No. MCH100106) provides information on the percentage of cells in each phase of cell cycle. As cells progress in the cell cycle, the DNA content changes. Cells in different phases can be distinguished on basis of DNA content. The Muse® Cell Cycle reagent contains a premixed cocktail with propidium iodide, a fluorescent DNA intercalating dye which can be used to measure DNA content in single cells. Cells with different DNA content can be distinguished on the Muse® Cell Analyzer.

The Muse® cell analyzer enabled rapid comparison of the mechanism of action of three compounds. Nocodazole, a microtubule disruptor, arrests cells in G2/M phase. Etoposide, a DNA topoisomerase I inhibitor, impacts cell cycle before its effects on apoptosis. Staurosporine, an apoptosis inducer, also shows G2/M arrest at low concentrations.



Obtaining sophisticated, flow cytometric, multiparameter cell cycle information has never been easier. Like many Muse® assays, the cell cycle assay involves a simple mix-read-analyze protocol, with the analysis steps guided by an intuitive touchscreen.



Simple, rapid flow cytometry—now at your side.

For details, visit: **www.emdmillipore.com/muse**

Notes

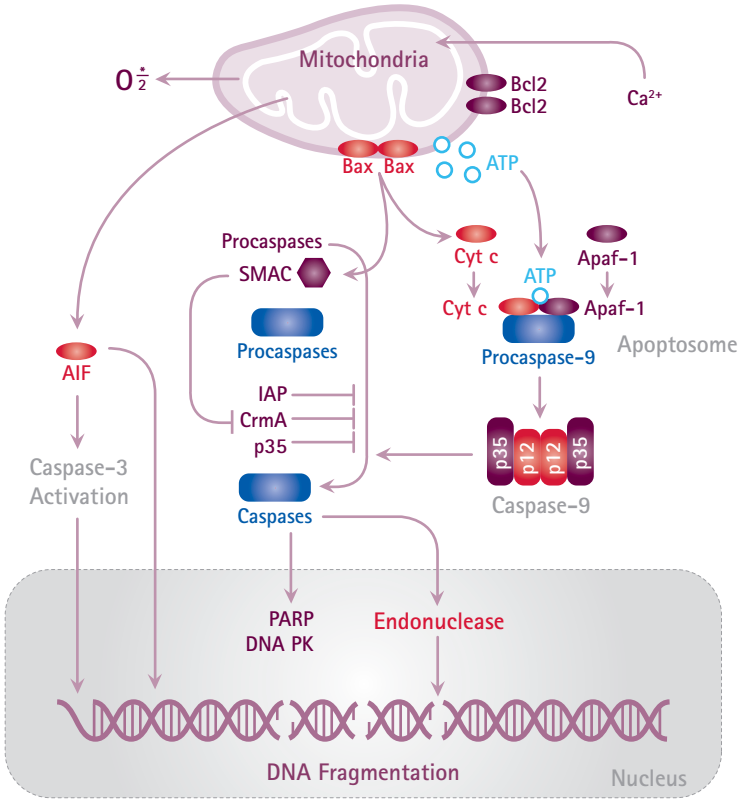
Evading Apoptosis

All cells are sensitive to proapoptotic signals; how can cancer cells be selectively induced to die?

Cancer cells evade apoptosis via multiple mechanisms. Most commonly, they exhibit mutations or deletions of tumor suppressor gene, TP53. Also, tumors may have diminished function of proapoptotic proteins, such as Bax or Puma, and they may show overexpression of anti-apoptotic Bcl2 family proteins.

Many cytotoxic antibiotics, including doxorubicin, bleomycin, and idarubicin, promote apoptosis in cancer cells by causing DNA damage. However, because these drugs broadly target DNA, they have many side effects. To avoid side effects, drugs must be developed to target proapoptotic molecules that are differentially expressed between tumor and normal tissues.

Recently, miRNAs have been identified that appear to regulate apoptosis and may be expressed in tumors but not in normal tissue. This discovery may yet open an avenue to developing therapeutic anti-miRs to promote death of tumor cells.



Apoptotic signaling begins with mitochondrial depolarization and ends with DNA fragmentation.

Featured Publications

1. Christensen LL et al. Functional screening identifies miRNAs influencing apoptosis and proliferation in colorectal cancer. PLoS One. 2014 Jun 3;9(6):e96767.
2. Wang WJ et al. Orphan nuclear receptor TR3 acts in autophagic cell death via mitochondrial signaling pathway. Nat Chem Biol. 2014 Feb;10(2):133-40.
3. Kreso A, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014 Jan;20(1):29-36.

Apoptosis Detection

Featured Technique:

Caspase Detection

A central component of the apoptotic process is a cascade of proteolytic enzymes called caspases. Caspases participate in a series of reactions that are triggered in response to pro-apoptotic signals and result in the cleavage of protein substrates, causing the disassembly of the cell. Caspases have been identified in organisms ranging from *C. elegans* to humans. In common with other proteases, caspases are synthesized as precursors that undergo proteolytic maturation, either autocatalytically or in a cascade by enzymes with similar specificity.

Caspase enzymes specifically recognize a 4 or 5 amino acid sequence on the target substrate which necessarily includes an aspartic acid residue. This residue is the target for cleavage, which occurs at the carbonyl end of the aspartic acid residue. Caspases can be detected via immunoprecipitation, immunoblotting techniques using caspase specific antibodies, or by employing fluorochrome substrates which become fluorescent upon cleavage by the caspase.

EMD Millipore provides a wide range of caspase antibodies, inhibitors, proteins, and assays to help detect and study caspase activation in apoptotic cells.

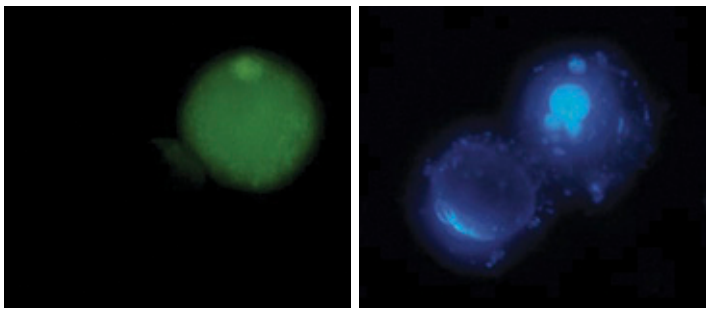
Featured Solution:

CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Fluorescein

(Catalog No. APT400)

EMD Millipore's *In Situ* Caspase Detection Kits use a novel approach to detect active caspases. The methodology is based on Fluorochrome Inhibitors of Caspases (FLICA). The inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the inhibitor binds covalently to the active caspase.

The CaspaTag™ Pan-Caspase *In Situ* Fluorescein Assay Kit uses a carboxyfluorescein-labeled fluoromethyl ketone peptide inhibitor of caspase (FAM-VAD-FMK), which produces a green fluorescence. When added to a population of cells, the FAM-VAD-FMK probe enters each cell and covalently binds to a reactive cysteine residue that resides on the large subunit of the active caspase heterodimer, thereby inhibiting further enzymatic activity. The bound labeled reagent is retained within the cell, while any unbound reagent will diffuse out of the cell and is washed away. The green fluorescent signal is a direct measure of the amount of active caspase present in the cell at the time the reagent was added. Cells that contain the bound labeled reagent can be analyzed by 96-well plate-based fluorometry, fluorescence microscopy, or flow cytometry.



Suspension cells were incubated with 1 mM staurosporine for 3 hours at 37°C to induce apoptosis. Cells were labeled with FAM-VAD-FMK and subsequently with Hoechst™ stain. Caspase activity in the left image was detected in one cell using a band pass filter. The second cell is not visible and therefore not apoptotic. Nuclear staining in the right image was revealed using a UV filter.

Technical Tip

Test your cancer cell model for both early apoptosis (mitochondrial membrane potential or Annexin V binding) and late apoptosis (DNA fragmentation). The relative activity of each step can reveal the model's Achilles' heel.

The Hallmark & Drug Development

PTC-209, a research compound, was observed to induce apoptosis in leukemia and colorectal cancer-initiating cells, which are a small subset of the entire tumor cell population. This compound works by blocking the self-renewal factor, BMI-1.

Featured Cancer: Ovarian Cancer

Many ovarian cancers lack p53 and are thus able to evade apoptosis. In a recent study, p53 was added to mitochondria in ovarian cancer cells by fusing p53 to peptides derived from mitochondrial proteins Bak and Bax.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Apoptosis:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-Bcl2, clone 100	05-729
	Anti-Cytochrome c, clone 7H8.2C12	MAB1800
	Anti-Annexin A1	ABC151
	Anti-Annexin A2	ABC152
	Anti-Annexin A4, clone PA351-29.1.3	MABN480
	Anti-ANXA6	ABC404
	Anti-Annexin IV	ABC885
	Anti-Annexin I, clone 6E4/3	MAB3773
	Anti-Apaf-1, rabbit monoclonal, clone E38	04-579
	Anti-Bax (NT)	ABC11
	Anti-Bax	AB2915
	Anti-Bcl-2	04-436
	Anti-Bcl-2	05-826
	Anti-Bim, internal epitope, pan-Bim isoforms	AB17003
	Anti-Bim, clone 14A8	MAB17001-50UG
	Anti-Caspase 3	06-735
	Anti-Caspase 3	ABC495
	Anti-Caspase3 (active form), clone 3D9.3	MAB10753
	Anti-Caspase 8	AB1879
	Anti-Caspase 8	MAB4708
	Anti-Caspase 8	06-775
	Anti-Caspase 9, clone 96-2-22	05-572
	Anti-Caspase 9, clone 2-23	MAB4709
	Anti-Caspase 9, active (cleaved)	AB3629
	Anti-Caspase-9 (Pro/p35), Rabbit Monoclonal	04-444
	Anti-Endonuclease G	ABC30
	Anti-Mcl-1	AB2910
	Anti-Mcl-1, clone RC13	MABC43
	Anti-Noxa	ABC50
	Anti-Noxa1	ABC117
	Anti-VDAC1, clone N152B/23	MABN504

For a complete solution, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Apoptosis:

Research Solutions	Description	Catalog No.
Related Proteins	Bcl-xL $BH4_{4-23}$, Human, Cell-Permeable	197217
	Caspase-3, Human, Recombinant, *E. coli*	235417
	Hexokinase II VDAC Binding Domain Peptide, Cell-Permeable	376816
Kits and Assays	ApopNexin™ Annexin V FITC Apoptosis Kit	APT750
	TUNEL Apoptosis Detection Kit	17-141
	CaspSCREEN™, non-adherent cells only	APT105
	CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Fluorescein	APT400
	CaspaTag™ Caspase 3,7 *In Situ* Assay Kit, Fluorescein	APT403
	CaspaTag™ Caspase 8 *In Situ* Assay Kit, Fluorescein	APT408
	CaspaTag™ Caspase 9 *In Situ* Assay Kit, Fluorescein	APT409
	CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Fluorescein	APT420
	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
	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
Small Molecules	Apoptosis Inhibitor	178488
	Apoptosis Inhibitor II, NS3694	178494
	Bax Activator, BAM7	196800
	Bax Channel Blocker	196805
	Bax-Inhibiting Peptide, V5	196810
	Bcl-2 Inhibitor	197330
	Bcl-2 Inhibitor II, YC137	197331
	Bcl-2 Inhibitor VI, ABT737	197333
	BMI-1 Expression Inhibitor, PTC-209	530154
	Caspase Inhibitor Set II	218772

For a complete solution, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Apoptosis:

Research Solutions	Description	Catalog No.
Small Molecules (continued)	Caspase Modulator I, 1541	264157
	Caspase Modulator II, 1541B	264158
	Caspase-3/7 Inhibitor II	218832
	DNA-PK Inhibitor	260960
	InhibitorSelect™ EGFR Signaling Pathway Panel	324839
	InhibitorSelect™ JAK/STAT Signaling Pathway Panel	420138
	InhibitorSelect™ MAPK Signaling Pathway Panel	444189
	InhibitorSelect™ NF-kB Signaling Pathway Panel	481487
	InhibitorSelect™ Tyrosine Kinase & Phosphatase Library I	539747
	InhibitorSelect™ WNT Signaling Pathway Panel	681666
	InSolution™ Caspase Inhibitor VI	219011
	InSolution™ Caspase-8 Inhibitor II	218840
	InSolution™ Caspase-9 Inhibitor I	218841
	JNK Inhibitor XIII, SR-3306	420147
	Phosphatase Inhibitor Cocktail Set II, Lyophilized	524636
	PLD Inhibitor, FIPI	528245

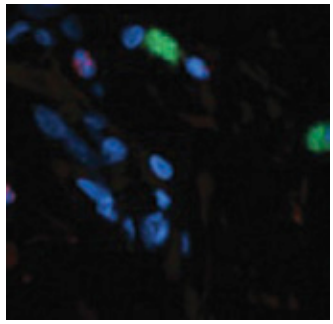
For a complete solution, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

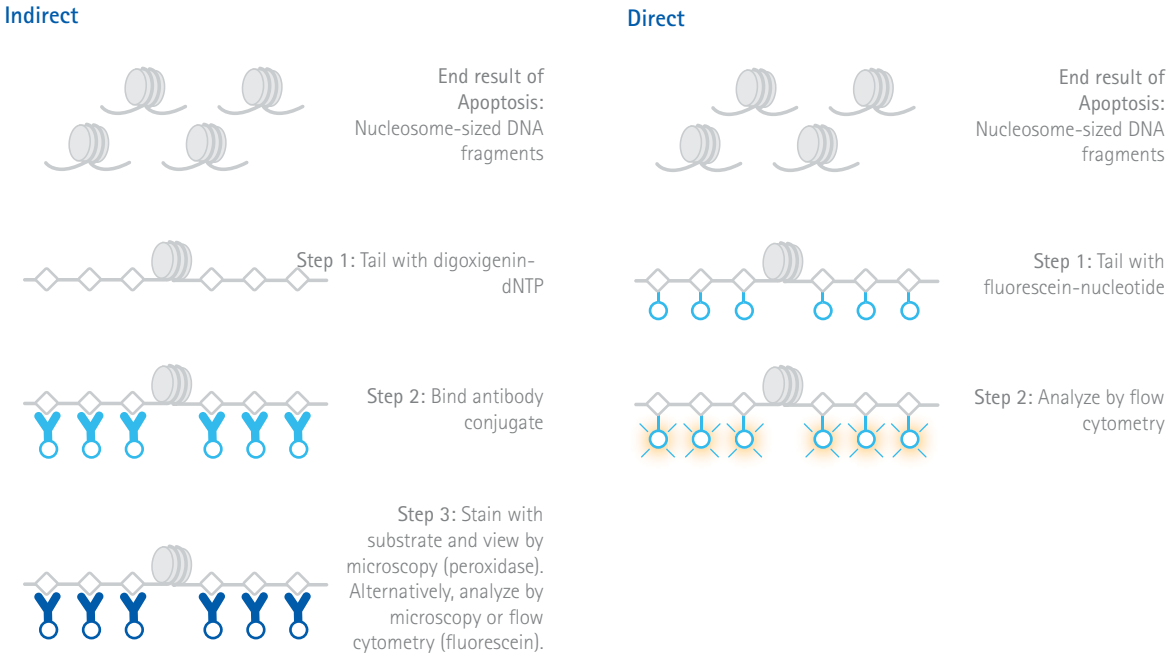
Sensitive detection of DNA fragmentation: ApopTag™ TUNEL assays

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.



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).



Measure the products of DNA fragmentation either indirectly or directly with ApopTag™ fluorescent TUNEL assays.

Choose the perfect TUNEL assay from our wide range of kits.

For details, visit: **www.emdmillipore.com/apoptosis**

EARLY STAGE

LATE STAGE

Apoptosis Cell-Based Assays From EMD Millipore

Hallmark Apoptotic Event	Concept	EMD 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™ assay	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

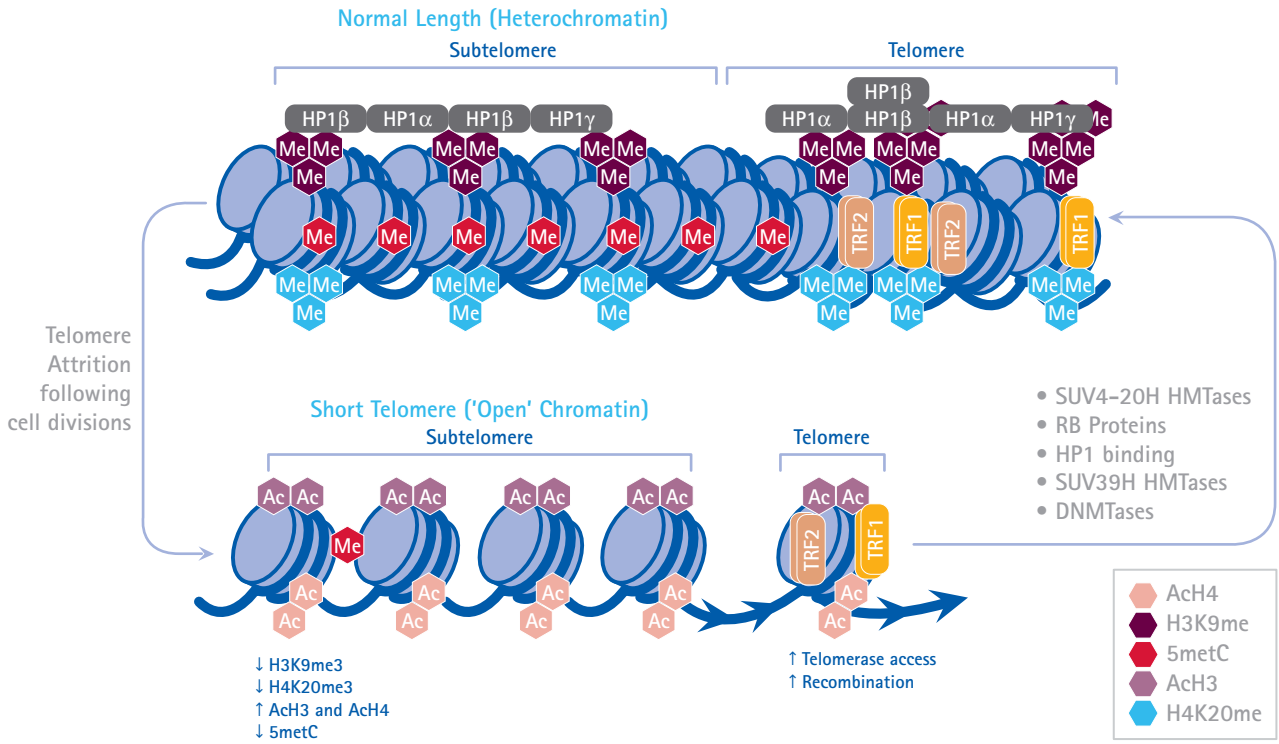
Application	Advantages	Catalog No.
Immunofluorescence	**Effective:** Classic model	APT750
Cytometry	**Versatile:** Detection options	
Flow Cytometry	**Effective:** Classic model	4500-0450, 4500-0455
	Versatile: Detection options	
Immunohistochemistry	**Sensitive:** Highly specific marker	05-719, 16-256
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	

Limitless Replicative Potential

Advances in epigenetics analysis has broadened our picture of telomere regulation beyond TERT.

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, as a built-in tumor suppression mechanism.

However, 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.



Chromatin modifications and multiprotein complexes regulating telomere length. (Adapted from Blasco, Nature Reviews Genetics 8, 299-309 (2007))

Featured Publications

1. Christensen LL et al. Functional screening identifies miRNAs influencing apoptosis and proliferation in colorectal cancer. PLoS One. 2014 Jun 3;9(6):e96767.
2. Koh CM, et al. Telomerase regulates MYC-driven oncogenesis independent of its reverse transcriptase activity. J Clin Invest. 2015 May;125(5):2109-22.
3. Novo CL, et al. A new role for histone deacetylase 5 in the maintenance of long telomeres. FASEB J. 2013 Sep;27(9):3632-42.

Replication Research

Featured Technique:

Validated Antibodies for Chromatin Immunoprecipitation

Antibody recognition in the context of chromatin can differ from other immunoassays. Avoid chromatin immunoprecipitation (ChIP) failure due to poor antibody performance by using ChIPAb+™ antibodies. Each lot is individually validated and tested for ChIP. Even when using a ChIP-grade antibody, detection conditions, including PCR primer quality, might affect your analysis. Each ChIPAb+™ antibody set includes control primers (every lot tested by qPCR) to validate your ChIP results. The ChIPAb+™ set also includes a negative control antibody to confirm specificity of the reaction.

ChIP-qualified Antibodies

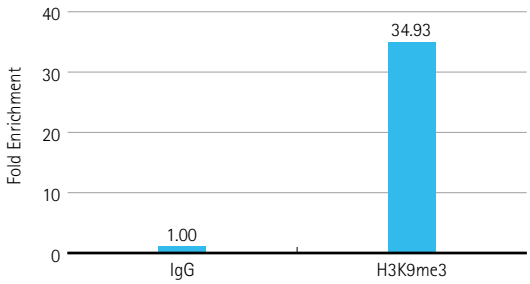
To complement our ChIPAb+™ validated antibody/primer sets, EMD Millipore also offers a wide selection of ChIP-qualified antibodies against modified and unmodified histones, transcription factors, and other key chromatin associated proteins.

Featured Solution:

ChIPAb+™ Trimethyl-Histone H3 (Lys9)

(Catalog 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 EMD Millipore's broad range of specific, highly validated antibodies and assays for epigenetic signaling pathways.



Sonicated chromatin prepared from 3x10^6 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.

Technical Tip

Using chromatin immunoprecipitation? Over cross-linking may result in low recovery of DNA. Long incubation in formaldehyde may mask epitopes required for recognition by ChIP antibody. This can be especially problematic if you are using a monoclonal antibody. Over cross-linking may also result in the formation of complexes that are resistant to sonication. Optimize cross-linking steps: the final concentration of formaldehyde should be 1%, and you should determine the most effective cross-linking time before proceeding with the experiment.

The Hallmark & Drug Development

Imetelstat, a telomerase inhibitor, is currently being developed to treat blood cancers. Although initial studies showed high liver toxicity, the drug could be successful if the toxicity is managed and combination therapies employed to boost efficacy.

Featured Cancer: Glioma

Certain genetic variants of the genes encoding the protein and RNA components of telomerase, TERT and TERC, have been linked with increased risk of the brain cancer, glioma. However, individuals carrying these variants may also enjoy improved health in general!

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Replication Signaling:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-acetyl-Histone H3 (Lys14), clone 13HH3-1A5	MABE351
	Anti-acetyl-Histone H4 (Lys91)	ABE361
	Anti-Bmi-1, clone 10C7.2	MAB4376
	Anti-Bmi-1, clone DC9	05-1322
	Anti-Bmi1, clone AF27	05-1321
	Anti-dimethyl Histone H3 (Lys9), clone CMA307	05-1249
	Anti-dimethyl-Histone H4 (Lys20), clone 6G7/H4	05-672
	Anti-HDAC5	07-045
	Anti-HDAC9, clone LH/JC2	05-897
	Anti-monomethyl Histone H3 (Lys9)	ABE101
	Anti-monomethyl Histone H4 (Lys20)	07-1570
	Anti-phospho-Histone H2A.X (Ser139), clone JBW301	05-636
	Anti-phospho-Histone H2B (Ser14)	07-191
	Anti-Sonic Hedgehog	06-1106
	Anti-Sonic Hedgehog (C-product), clone EP1190Y, Rabbit	04-971
	Anti-trimethyl Histone H3 (Lys9)	07-442
	Anti-trimethyl Histone H3 (Lys9), Alexa Fluor® 488 Conjugate	07-442-AF488
	Anti-trimethyl Histone H3 (Lys9), Alexa Fluor® 647 Conjugate	07-442-AF647
	Anti-trimethyl-Histone H4 (Lys20), rabbit monoclonal	04-079
	ChIPAb+™ Trimethyl-Histone H3 (Lys9) Antibody/Primer Set	17-625
Related Proteins	Aurora A, active purified kinase	14-511
Kits and Assays	CpG MethylQuest™ DNA Isolation Kit	17-10035
	CpGenome™ Turbo Bisulfite Modification Kit	S7847
	CpG WIZ® ERα Amplification Kit	S7815
	TRAPeze® Telomerase Detection Kit	S7700
	BrdU Immunohistochemistry System	HCS30
	Senescence Detection Kit	QIA117

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Replication Signaling:

Research Solutions	Description	Catalog No.
Small Molecules	Aurora Kinase/Cdk Inhibitor	189406
	BMI-1 Expression Inhibitor, PTC-209	530154
	Daminozide	251635
	DNA Methyltransferase Inhibitor RG108	260920
	Grp94 Inhibitor, PU-WS13	508307
	HDAC6 Inhibitor II, BRD9757	505760
	HDAC6 Inhibitor III	508194
	HMTase Inhibitor XI, RSC133	500509
	InSolution™ Chk2 Inhibitor II	220491
	InSolution™ EZH2 Inhibitor, DZNep	506069
	InSolution™ SRT1720, HCl	530748
	PARP Inhibitor Set II	505328
	PIPER	528120
	Purmorphamine	540220
	Remodelin	531066
	RPA Inhibitor, TDRL-505	530535
	SIRT1/2/3 pan Inhibitor	505479
	SIRT2 Inhibitor, AEM2	530648
	Smoothened Agonist, SAG	566660
	Splitomicin	567750
	Telomerase Inhibitor III, Sodium Salt	581004
	Telomerase Inhibitor IX	581011
	Telomerase Inhibitor X, BIBR1532	508839
	Topoisomerase II Inhibitor, BNS-22	614853
	Trichostatin A, Streptomyces sp.	647925
	Windorphen	509164
	Zebularine	691400

For a complete selection, visit: www.emdmillipore.com

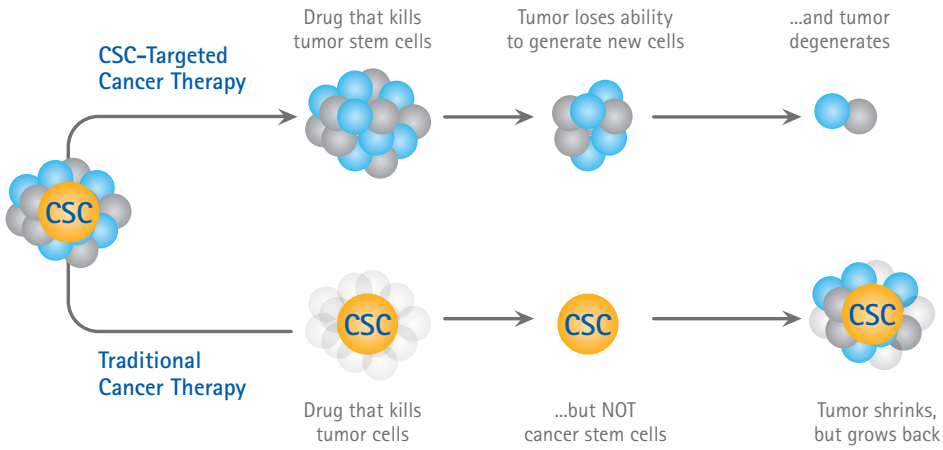
TECHNOLOGY HIGHLIGHT

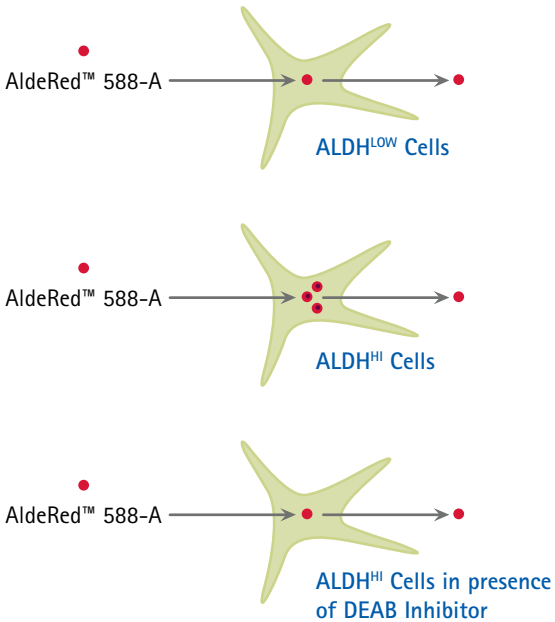
AldeRed™ ALDH Detection Kit

Live cancer stem cell identification, dual labeling on a GFP background

High ALDH activity serves as a universal marker of stem cells, both normal and malignant. Cells can be identified and isolated based upon the enzymatic activity of ALDH, a detoxifying enzyme responsible for oxidation of hazardous aldehyde byproducts. The marker ALDH has been used to isolate cancer stem cells from various human malignancies including bladder, breast, cervical, colon, head and neck, liver, lung, pancreas, prostate, and ovary.

Research over recent years have indicated the existence of CSCs in nearly all solid tumors at varying frequencies. A number of cell surface markers have proved useful for isolation and enrichment for CSCs including CD133, CD44, CD24, EpCAM, THY-1 (CD90), ATP-binding cassette B5 (ABCB5), CD200, and the enzyme aldehyde dehydrogenase (ALDH).





The AldeRed™ ALDH Detection Kit provides cancer and stem cell scientists with new capabilities for live cell isolation and characterization. The AldeRed™ reagent is a red-shifted fluorescent substrate for aldehyde dehydrogenase (ALDH), allowing cells to be identified and isolated by flow cytometry with concurrent use of green fluorescent cell lines, antibodies, transgenic animals, and reporter assays (Minn, 2014).

Features and Benefits

- Red-shifted assay leaves green channel available for further experimentation
- Live stem cell identification enables flow sorting of rare cell populations
- Rapid enzymatic assay protocol

Description	Catalog No.
AldeRed™ ALDH Detection Kit	SCR150

For for information, visit: **www.emdmillipore.com/aldered**

Notes

Sustained Angiogenesis

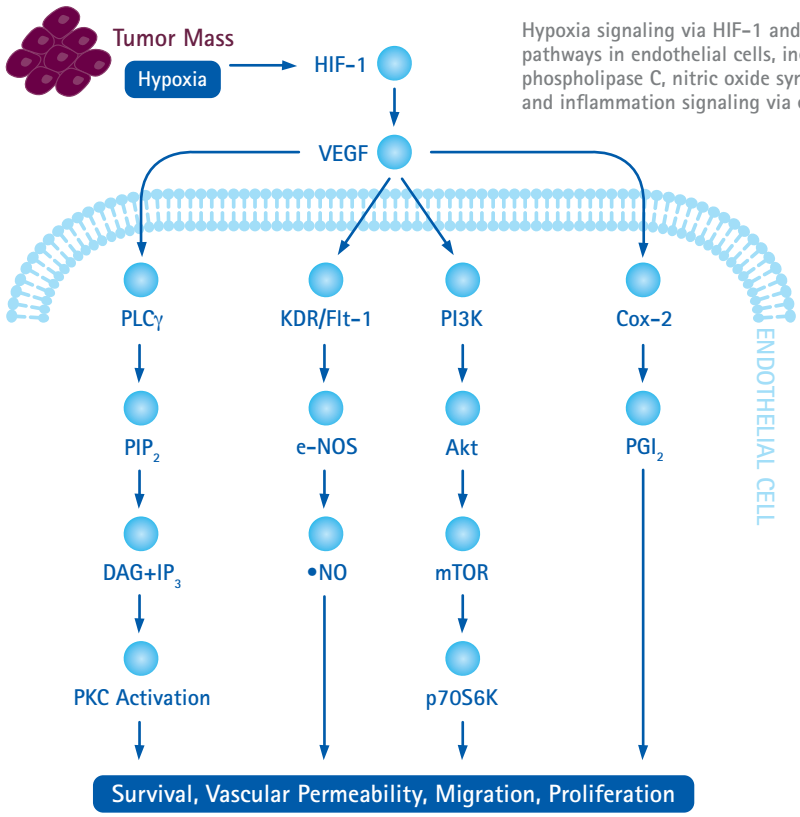
Inside a tumor, lack of oxygen drives new blood vessel growth.

The hypoxic environment inside a tumor activates hypoxia-inducible factor 1 (HIF-1), a transcription factor, to drive the expression of vascular endothelial growth factor (VEGF). VEGF is secreted from the tumor and binds to receptors on endothelial cells, activating signal pathways that cause the endothelial cells proliferate to form new blood vessels.

Because both angiogenesis and inflammation are dependent on activation of stromal cells and on cytokines, chemokines, growth factors, prostaglandins, and several proteases, many disease states that involve chronic inflammation can result in tumorigenesis.

Although most drugs designed to block angiogenesis result in extending survival only by months, these drugs may yet be of value in the context of combination therapies. As more molecular details of angiogenic pathways in cancer are uncovered, new therapeutic strategies also emerge. One new therapeutic direction involves targeting the metabolism of tumor-associated endothelial cells.

VEGF signaling in angiogenesis



Hypoxia signaling via HIF-1 and VEGF activates multiple pathways in endothelial cells, including lipid signaling via phospholipase C, nitric oxide synthase, PI3-kinase/Akt/mTOR, and inflammation signaling via cyclooxygenase-2 (Cox-2).

Featured Publications

1. Arendt LM, et al. Obesity promotes breast cancer by CCL2-mediated macrophage recruitment and angiogenesis. Cancer Res. 2013 Oct 1;73(19):6080-93.
2. Yeo EJ, et al. Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. Cancer Res. 2014 Jun 1;74(11):2962-73.
3. Zecchin A, et al. Endothelial cells and cancer cells: metabolic partners in crime? Curr Opin Hematol. 2015 May;22(3):234-42.

Studying Angiogenesis

Featured Technique:

Tube Formation

The process of generating new capillary blood vessels, angiogenesis, can be studies *in vitro* by assaying the formation of tube like structures by endothelial cells. When cultured in plates coated with a matrix containing basement proteins, the endothelial cells rapidly align and form hollow tube-like structures. Tube formation assays can be used for studying both the induction and the inhibition of endothelial cell function. For assaying inhibitors or stimulators of tube formation, the endothelial cell suspension is pre-mixed with different concentrations of the inhibitor or stimulator to be tested before being plated. Tube formation is a multi-step process involving cell adhesion, migration, differentiation, and growth.

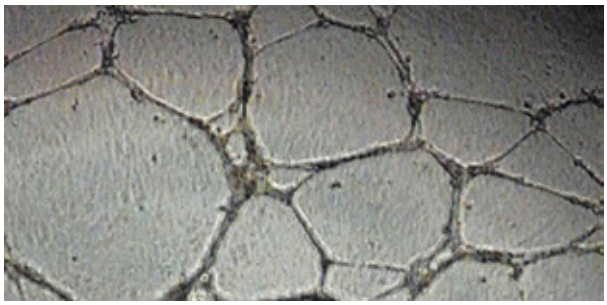
Featured Solution:

In Vitro Angiogenesis Assay Kit

(Catalog No. ECM625)

EMD Millipore's *In Vitro* Angiogenesis Assay Kit represents a simple model of angiogenesis in which the induction or inhibition of tube formation by exogenous signals can be easily monitored. The assay can be used to monitor the extent of tube assembly in various endothelial cells, e.g. human umbilical vein cells (HUVEC) or bovine capillary endothelial (BCE) cells.

The *In Vitro* Angiogenesis Assay Kit provides a convenient system for evaluation of tube formation by endothelial cells in a convenient 96-well format. The plates are pre-coated with ECMatrix™, a solid gel of basement proteins prepared from the Engelbreth Holm-Swarm (EHS) mouse tumor, and contains various growth factors (TGF-β, FGF) and proteolytic enzymes (plasminogen, tPA, MMPs) that occur normally in EHS tumors. It is optimized for maximal tube-formation.



HUVEC incubated 6–10 hours at 37°C on ECMatrix™.
Activated endothelial cells form cellular networks (mesh-like structures) from capillary tubes sprouting into the stromal space (see figure). The formation of these cellular networks is a dynamic process, starting with cell migration and alignment, followed by the development of capillary tubes, sprouting of new capillaries, and finally the formation of the cellular networks. Although the *In Vitro* Angiogenesis Assay Kit is designed as a qualitative assay, it is possible to quantitate to some degree the extent to which cellular networks have formed. One can quantitate based on pattern recognition, branch points, tube length, and visualizing cell-tubes.

Technical Tip

When measuring angiogenesis *in vitro*, stain blood vessels using conjugated antibodies to von Willebrand factor (vWF) and CD31, which bind selectively to the surface of endothelial cells.

The Hallmark & Drug Development

Bevacizumab was the first approved drug that targeted angiogenesis. It blocks the binding of VEGF to VEGF receptors, and has been shown to extend survival of metastatic colon cancer patients.

Featured Cancer: Renal Cell Carcinoma

Many renal cell carcinomas have inactivating mutations in the von Hippel Landau (vHL) gene, which results in overactive HIF-1. As a result, anti-angiogenic drugs, such as bevacizumab, sunitinib, and sorafenib are frequently used to treat renal cell carcinoma.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Angiogenesis:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-Angiomotin	ABS1024
	Anti-Angiopoietin-1	AB10516
	Anti-Angiopoietin-2, NT	AB3121
	Anti-Angptl2	ABC85
	Anti-Angptl3	ABC83
	Anti-Angptl6	ABC84
	Anti-Brain-Specific Angiogenesis Inhibitor 2	AB9413
	Anti-Brain-Specific Angiogenesis Inhibitor 3	AB9125
	Anti-Endostatin	05-579
	Anti-FGF-2/basic FGF (neutralizing), clone bFM-1	05-117
	Anti-PDGF, neutralizing	06-127
	Anti-PDGFR	05-1135
	Anti-phospho-EGFR (Tyr1069)	09-310
	Anti-phospho-PDGFRα(Tyr754)	ABS150
	Anti-phospho-Tie2 (Ser1119)	ABS219
	Anti-phospho-VEGFR2 (Tyr1212), clone 12A10.1	MABS191
	Anti-TGFBI	ABT358
	Anti-Tie-2, extracellular, clone 1E11DH	MAB1148
	Anti-Tie-2, extracellular, clone 4G8HE	MAB1149
	Anti-Tie2 Mouse mAb (Ab33)	610205
	Anti-VEGF	07-1420
	Anti-VEGF Receptor-3, extracellular domain, clone 9D9F9	MAB3757
	Anti-VEGF, clone JH	05-443
	Anti-VEGF165	ABS282
	Anti-vWF (von Willebrand Factor)	AB7356
	Anti-vWF (von Willebrand Factor), clone GMA-022	05-861
	PhosphoDetect™ Anti-Tie2 (Ab-1) (pTyr1102/1108) Rabbit pAb	PC449
	PhosphoDetect™ Anti-Tie2 (pTyr 1108/1117) (Ab-2) Rabbit pAb	PC450

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Angiogenesis:

Research Solutions	Description	Catalog No.
Assays and Kits	*In Vitro* Vascular Permeability Assay (24-well)	ECM644
	In Vitro Vascular Permeability Assays (96-well)	ECM642
	In Vitro Angiogenesis Assay Kit	ECM625
	Fibrin *In Vitro* Angiogenesis Assay	ECM630
	MILLIPLEX® MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel 1	HAGP1MAG-12K
	MILLIPLEX® MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel 2	HANG2MAG-12K
	ChemiScreen™ CXCR2 Chemokine Receptor Membrane Preps	HTS002M
	Ready-to-Assay™ EP2 Prostanoid Receptor Frozen Cells	HTS185RTA
	Blood Vessel Staining Kit	ECM590
	Blood Vessel Staining Kit, Alkaline Phosphatase conjugated	ECM595
	Endothelial Cell Characterization Kit	SCR023
Small Molecules	InhibitorSelect™ VEGF Signaling Pathway Panel	676502
	Amiloride, HCl	129876
	EF24	324510
	FGF-2 Inhibitor, NSC37204	341598
	Fumagillin, *Aspergillus fumigatus*	344845
	4-Hydroxyphenylretinamide	390900
	2-Methoxyestradiol	454180
	Mifepristone	475838
	Shikonin	565850
	(+/-)-Thalidomide	585970
	Tranilast	616400
	VEGF Inhibitor, CBP-P11	676496
	VEGF Receptor 2 Kinase Inhibitor I	676480
	VEGF Receptor 2/Flt3/c-Kit Inhibitor	676500
	TAS-301	608050
	VEGF Inhibitor, CBO-P11	676496
	Withaferin A, *Withania somnifera*	681535

For a complete selection, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

Correlation of angiogenesis biomarkers with early metastatic progression in NSCLC as determined using a multiplexed immunoassay kit

MILLIPLEX® MAP Human Angiogenesis Panel 1

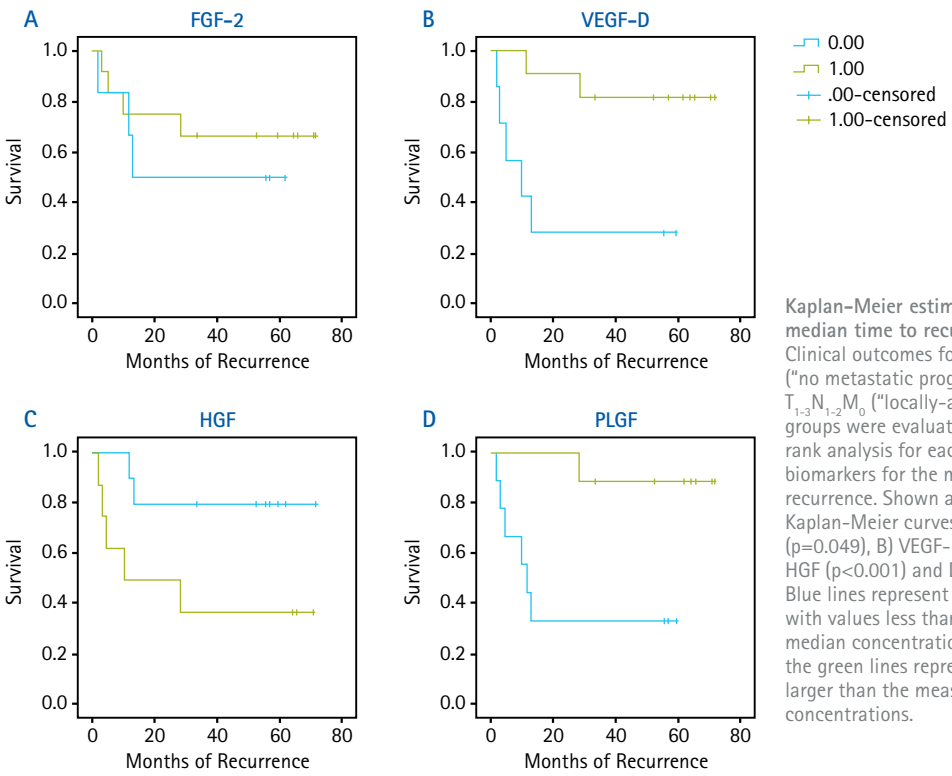
- Angiopoietin-2
- BMP-9
- EGF
- Endoglin
- Endothelin-1
- FGF-1
- FGF-2
- Follistatin
- G-CSF
- HB-EGF
- HGF
- IL-8
- Leptin
- PLGF
- VEGF-A
- VEGF-C
- VEGF-D

MILLIPLEX® MAP Human Angiogenesis Panel 2

- Angiostatin
- sAXL
- sc-Kit/SCFR
- sHer2
- sHer3
- sE-Selectin
- sHGFR/c-Met
- Tenascin C
- PDGF-AB/BB
- sIL-6Ra
- sTie-2
- sThrombospondin-2
- sNeuropilin-1
- sEGFR
- suPAR
- sVEGFR1
- sVEGFR2
- sVEGFR3
- sPECAM-1/CD31
- Osteopontin

From an investigative and therapeutic standpoint, it is important to identify and subsequently target the growth factors or regulatory elements that play crucial roles in allowing angiogenic dysregulation. With the overwhelming numbers of these targets for potential investigation, it is a challenge to decide among them and efficiently quantitate multiple targets. Current detection assays are limited by minimal automation, low overall output and excessive cost. To quickly and affordably identify specific angiogenic processes, it is necessary to screen large panels of vascular analytes and growth factors with some level of automation or high throughput.

In collaboration with the laboratory of Jeffrey Borgia at Rush University Medical Center, we demonstrated the utility of the MILLIPLEX® MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel 1, based on the Luminex® xMAP® bead-based multiplexed assay platform, in the analysis of 17 targets involved in the angiogenesis pathway in a cohort of 38 patients with various stages of non-small cell lung cancer (NSCLC). Our objective was to determine if the MILLIPLEX® assay could accurately quantify known angiogenesis targets in a practical, high throughput fashion.



Kaplan-Meier estimates of the median time to recurrence. Clinical outcomes for the $T_{1-2}N_0M_0$ ("no metastatic progression") and $T_{1-3}N_{1-2}M_0$ ("locally-advanced") groups were evaluated via log-rank analysis for each of the 17 biomarkers for the median time to recurrence. Shown above are the Kaplan-Meier curves for A) FGF-2 (p=0.049), B) VEGF-D (p=0.009), C) HGF (p<0.001) and D) PLGF (0.010). Blue lines represent patients with values less than measured median concentrations, whereas the green lines represent values larger than the measured median concentrations.

Discover more multiplex and single protein immunoassays for circulating and intracellular cancer biomarkers.

Visit: **www.emdmillipore.com/bmia**

Notes

Metabolic Reprogramming

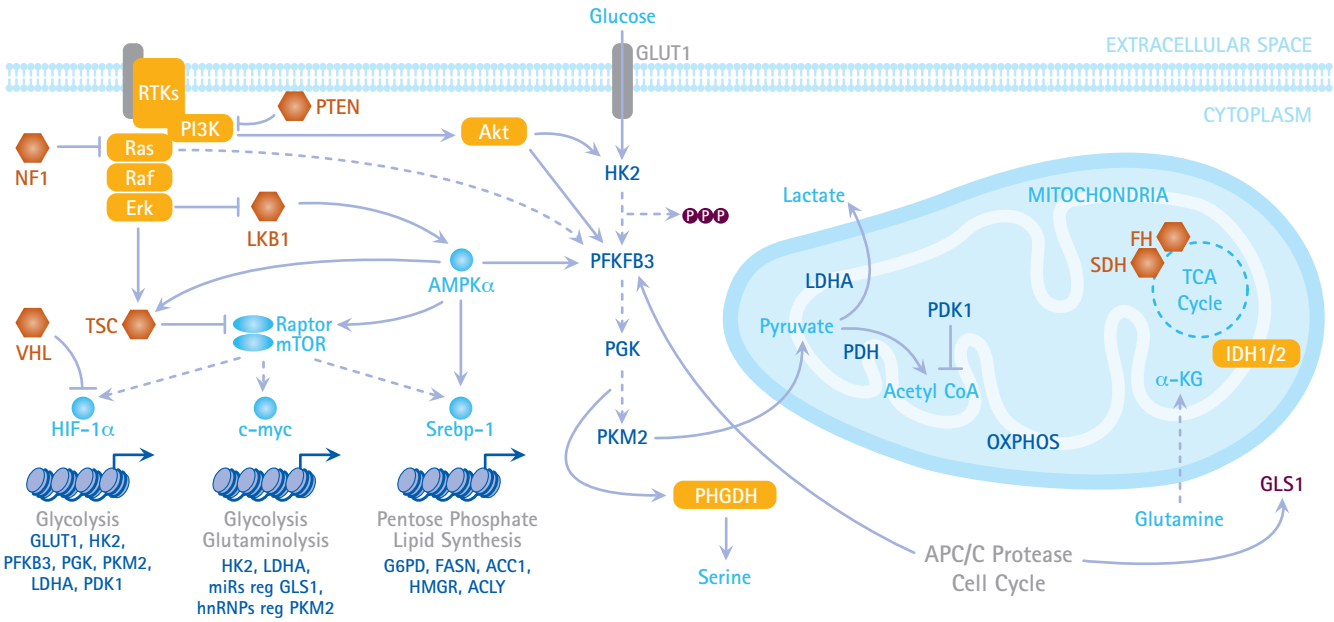
Tumor cells proliferate under duress, using glucose, glutamine, α-ketoglutarate, and one-carbon metabolism.

Rapidly proliferating cancer cells have extraordinary nutritional needs, and they undergo appropriately extraordinary adaptations to meet those needs. The most well-understood adaptation is the Warburg effect, in which tumor cells express a form of pyruvate kinase that enables them to maintain a glycolytic rate 200 times that of normal cells, providing energy even when mitochondria may be damaged.

In another metabolic adaptation, cancer cells express mutant isocitrate dehydrogenase (IDH), which convert α-ketoglutarate into 2-hydroxyglutarate. The latter metabolite activates angiogenesis via the HIF-1 pathway and also affects gene expression by repressing histone demethylases.

Glutamine is an important nitrogen source for starved cancer cells, enabling rapid protein and nucleic acid synthesis. cMyc, an oncogene amplified in many tumors, upregulates the expression of glutamine metabolism proteins, which induce autophagy and promotes tumor cell survival.

Most recently, studies of cancer cell metabolism have included one-carbon metabolism, fed by serine and glycine. These amino acids may contribute to histone methylation and pluripotency of cancer stem cells. Other molecules involved in one-carbon metabolism include folate, methionine, cysteine, and glutathione.



Oncogenes (orange) and tumor suppressors (brown hexagons) can both play complementary roles in reprogramming metabolic pathways of cancer cells to adapt to intense nutritional needs irrespective of oxygenation or mitochondrial damage.

Featured Publications

1. Kaelin WG Jr. DisABLing kidney cancers caused by fumarate hydratase mutations. Cancer Cell. 2014 Dec 8;26(6):779-80.
2. Izquierdo-Garcia JL, et al. Metabolic reprogramming in mutant IDH1 glioma cells. PLoS One. 2015 Feb 23;10(2):e0118781.
3. Bhutia YD, et al. Amino Acid Transporters in Cancer and Their Relevance to "Glutamine Addiction": Novel Targets for the Design of a New Class of Anticancer Drugs. Cancer Res. 2015 May 1;75(9):1782-8.

Metabolic Reprogramming

Featured Technique:

Metabolic Enzyme Immunodetection

Enzymes that catalyze metabolic reactions are critical to maintaining metabolic balance in cells. Changes in enzyme activity as well as changes in enzyme expression levels can indicate metabolic reprogramming. These changes can be detected using antibody-based assays, such as immunofluorescence, flow cytometry, and Luminex® bead-based assays.

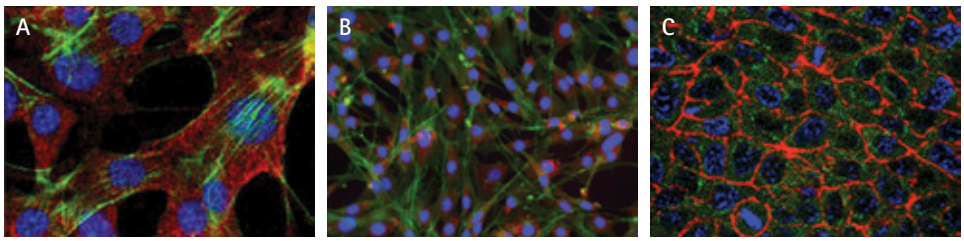
Featured Solution:

Anti-Glyceraldehyde-3-Phosphate Dehydrogenase Antibody, clone 6C5

(Unconjugated: MAB374; Alexa Fluor® 647 conjugate: MAB374-AF647; Alexa Fluor® 488 conjugate: MAB374-AF488)

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a ubiquitous glycolytic enzyme present in reasonably high levels in almost all tissues. As a housekeeping enzyme, it catalyzes the synthesis of 1,3-biphosphoglycerate, a high energy intermediate used for the synthesis of ATP. It is also involved in the initial stages of apoptosis or oxidative stress response where GAPDH is translocated to the nucleus. GAPDH has also been found to bind specifically to proteins implicated in the pathogenesis of a variety of neurodegenerative disorders, including β-amyloid precursor protein and huntingtin. GAPDH has also been identified as a potential target for nitric oxide (NO)-mediated cellular toxicity.

EMD Millipore offers specific and validated antibodies for GAPDH, based on the expertise of Upstate® and Chemicon®.



Immunofluorescent analysis using Anti-Glyceraldehyde-3-Phosphate Dehydrogenase, clone 6C5 antibodies. A: NIH/3T3 cells were labelled with unconjugated antibody (MAB374). Cytosolic staining was observed (Red). Actin filaments have been labeled with Alexa Fluor 488-Phalloidin (Green). B: NIH/3T3 cells were labelled with Alexa Fluor® 647 conjugated antibody (MAB374-AF647). Cytosolic staining was observed (Red). Actin filaments have been labeled with Phalloidin-Alexa Fluor® 488 (Green). C: A431 cells were labelled with Alexa Fluor® 488 conjugated antibody (MAB374-AF488). Cytosolic staining was observed (Green). Actin filaments have been labeled with Phalloidin-Rhodamine (Red). Nuclei are stained with DAPI (Blue).

Additional GAPDH Antibodies

Description	Catalog No.
Anti-GAPDH (CT), clone RM114	MABS819
Anti-GAPDH Mouse mAb (6C5)	CB1001
Anti-GAPDH	ABS16
Anti-GAPDH	AB2302
Anti-acetyl-GAPDH (Lys160)	07-2184
Milli-Mark® Anti-GAPDH-FITC, clone 6C5	FCMAB252F

Technical Tip

Performing live cell analysis? Antibodies must be preservative-free in live cell assays. Use diafiltration- or dialysis-based buffer exchange if necessary to remove preservatives.

The Hallmark & Drug Development

AG221, an early-stage drug that selectively inhibits IDH2, has shown promising results. In a phase I trial, 25 out of 45 leukemia patients had a complete or partial response to the drug.

Featured Cancer: Pancreatic Ductal Adenocarcinoma

Glutamine metabolism and autophagy are important pathways in the progression of pancreatic ductal adenocarcinoma. Current research focuses on identifying the key proteins involved; combination therapies to target both pathways may be required to treat this aggressive cancer.

RESEARCH TOPIC SPOTLIGHT

Autophagy

Autophagy is an intracellular catabolic pathway that 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.

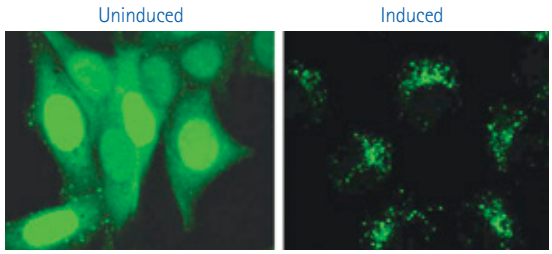
EMD Millipore is the leader in providing new, innovative products for studying autophagy mechanisms and identifying activators and inhibitors of autophagy.

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

EMD 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.



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.emdmillipore.com/autophagyvideo

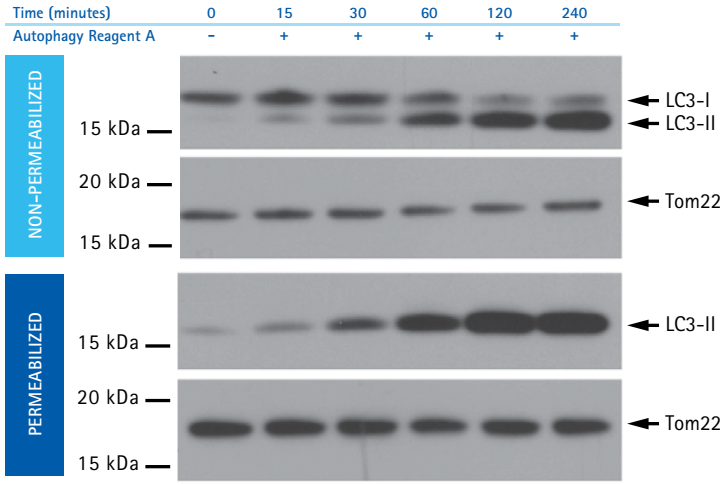
Description	Catalog No.
LentiBrite™ GFP-LC3 Lentiviral Biosensor	17-10193
LentiBrite™ RFP-LC3 Lentiviral Biosensor	17-10143
LentiBrite™ GFP-LC3 Control Mutant Lentiviral Biosensor	17-10189
LentiBrite™ RFP-LC3 Control Mutant Lentiviral Biosensor	17-10188
LentiBrite™ GFP-p62 Lentiviral Biosensor	17-10224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

RESEARCH TOPIC SPOTLIGHT

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

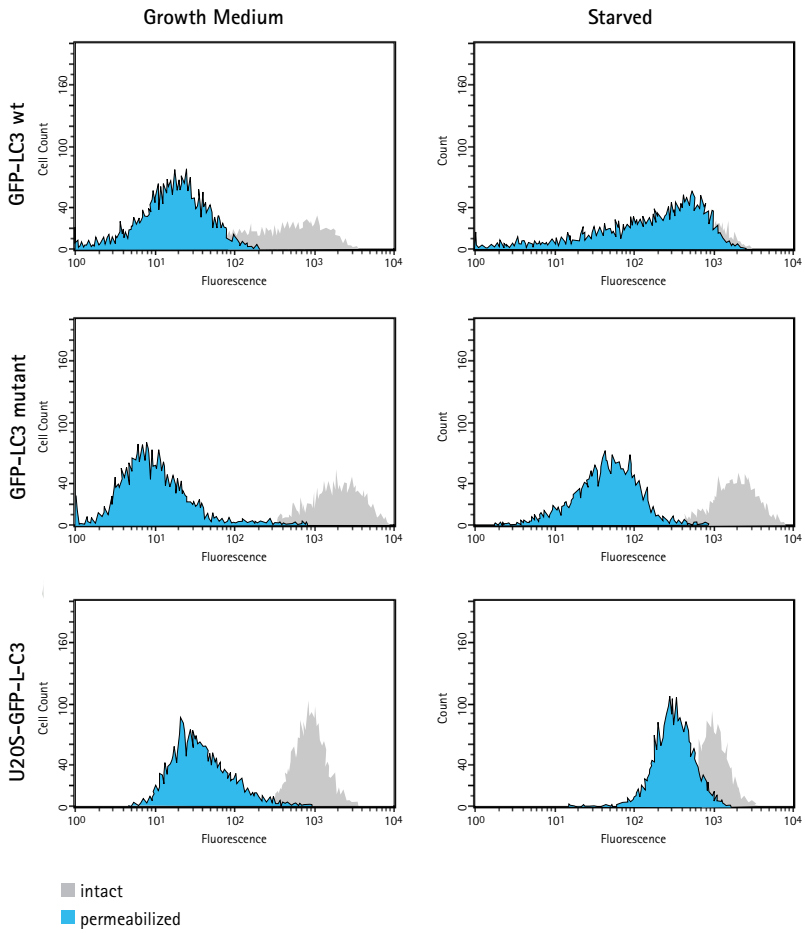


Non-enriched lysate and LC3-II-enriched protein fraction from HeLa cells were prepared with the LC3-II Enrichment Kit (Western Blot, Catalog No. 17-10232). Western blots of non-enriched lysates indicate the LC3-I signal decreases over time after induced autophagy, as the LC3-II signal increases. After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

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

RESEARCH TOPIC SPOTLIGHT

Related Products

Description	Applications	Catalog No.
Anti-ATG3	WB	AB2953
Anti-ATG5	WB, IHC	ABC14
Anti-ATG5	WB, ICC, IHC	AB15404P
Anti-ATG5, clone 177.19	WB, IP	MAB2605
Anti-ATG7	WB, IHC	AB10511
Anti-ATG10	WB. ICC. IHC	AB15408
Anti-ATG16L1	WB, IHC	ABC25
Anti-UVRAG	WB, ICC, IP	AB2960
Anti-mTOR, clone 21A12.2	WB, ICC	05-1564
Anti-phospho-mTOR (Ser2448)	WB	09-213
Anti-Beclin-1	WB, ICC, IHC	AB15417

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

Assays

Description	Catalog 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

Target	Product	Catalog No.
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
Autophagy Inducer	STF-62247	189497
Autophagy Stimulator	SMER28	573121
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

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Metabolism:

Research Solutions	Description	Catalog No.
Related Antibodies	PhosphoDetect™ Anti-PDH-E1α (pSer293) Rabbit pAb	AP1062
	PhosphoDetect™ Anti-PDH-E1α (pSer232) Rabbit pAb	AP1063
	PhosphoDetect™ Anti-PDH-E1α (pSer300) Rabbit pAb	AP1064
	Anti-BRCA1 (Ab-1) Mouse mAb (MS110)	OP92
	Anti-RRM2	ABC106
	Anti-IDH1	ABC1483
	Anti-IDH2	ABC57
	Anti-Isocitrate Dehydrogenase 1 (IDH1), clone RMab-3	MABC197
	Anti-Isocitrate Dehydrogenase 1 (IDH1)-R132S, clone SMab-1	MABC187
	Anti-PDK2	ABC755
	Anti-AMPKα1, rabbit monoclonal	04-323
	Anti-phospho-AMPKα (Thr172)	07-626
	Anti-AMPK α1	07-350
	Anti-AMPK α2	07-363
	Anti-AMPK α, clone 34.2	MABS1232
	Anti-PGC-1α, clone 4C1.3	ST1202
	Anti-PGC-1α, clone 1F3.9	ST1203
	Anti-PGC-1 β	ABC218
	Anti-PGC-1, C-Terminal (777-797), Rabbit pAb	516557
	Anti-Hypoxia Inducible Factor 1 α, clone H1α67	MAB5382
	Anti-Mitochondria, surface of intact mitochondria, clone 113-1	MAB1273
	Anti-PI3 Kinase, p110α	09-481
Assays and Kits	FlowCellect® Multi-STAT Activation Profiling Kit	FCCS025550
	FlowCellect® EGFR/STAT1 Activation Dual Detection Kit	FCCS025142
	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-1α MAPmate™ assay	46-665
	MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS)	H0XPSMAG-16K

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Metabolism:

Research Solutions	Description	Catalog No.
Small Molecules	3-Bromopyruvate	376817
	6-Benzylaminopurine	200241
	AICA-Riboside	123040
	AICA-Riboside 5'-Phosphate	123041
	AMPK Activator	171256
	AMPK Activator VI, RSVA314	171272
	b-Sitosterol	567152
	C75	341325
	FX11	427218
	Ghrelin, Human, Synthetic	494126
	Ghrelin, Rat, Mouse, Synthetic	494127
	HIF-1 Inhibitor	400083
	L-3,3',5-Triiodothyronine, Sodium Salt	64245
	PDK1 Inhibitor II	521276
	Phloretin	524488
	PKM2 Activator IV, TEPP-46	505487
	PKM2 Activator, DASA	550602
	WZB117	400036

For a complete selection, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

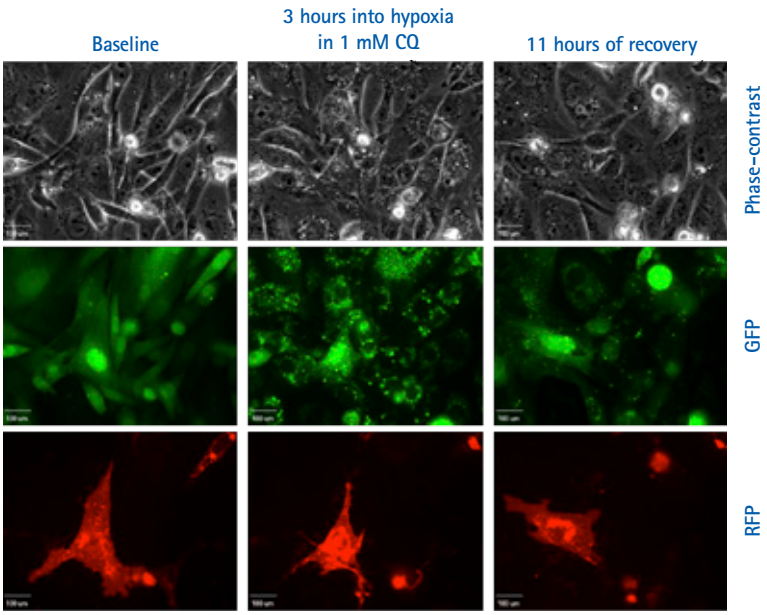
Visualizing autophagy in live cells while manipulating nutrients and gas composition: CellASIC® ONIX microfluidic platform

Tumor cells may use the autophagic pathway to promote survival. Here a live cell imaging assay using the CellASIC® ONIX microfluidic platform was used to monitor both the rate of autophagosome formation and changes in lysosomal degradative processes during autophagy. Cell lines stably expressing fluorescently-tagged markers specific for autophagosomes (LC3-GFP), were used in combination with microfluidic control of media and gas exchange to create starvation or hypoxia. Changes in LC3 levels (as measured by autophagosome counts) were monitored and quantified throughout culture duration by fluorescence microscopy.

Experimental manipulation of the cellular microenvironment is important when studying cancer cell behavior under stressed conditions, such as nutrient deprivation, hypoxia or drug exposure.

The CellASIC® ONIX microfluidic platform allows you to:

- Achieve control and measurement precision in autophagy assays
- Tightly control nutrient composition and delivery
- Program automated changes in gas mixtures
- Visualize real time autophagic changes in undisturbed cultures



Two-color imaging of transduced LAMP1-RFP/ LC3-GFP CHO reporter cells showing autophagosomes (green) and lysosomes (red) throughout the entire hypoxia-induced autophagy assay. Cells were first treated with mild hypoxia (3%) for 3 hours in the presence or absence of CQ, followed by the removal of the stressors and the treatment of normoxia and the growth medium for another 16 hours. An Olympus IX-71 inverted microscope was used for the entire process, and all images were taken under the 40x objective.

Take control with dynamic cell culture.

The CellASIC® platform consists of a microincubator controller that regulates heat, gas and flow to cell cultures via a manifold. Controlled by intuitive software and compatible with any microscope, the system enables easy programming of automated changes to conditions without any need to remove the cells from the microscope stage.

For details, visit: **www.emdmillipore.com/CellASIC**

Escape from Immune Control

Understanding and targeting the specific tumor microenvironment will be key to enabling immune cells to infiltrate and then attack a tumor.

After decades of being thwarted by a veritable shield raised by tumors protecting themselves from the innate immune system, cancer researchers have finally begun to discover how to redirect immunity against those tumor cells.

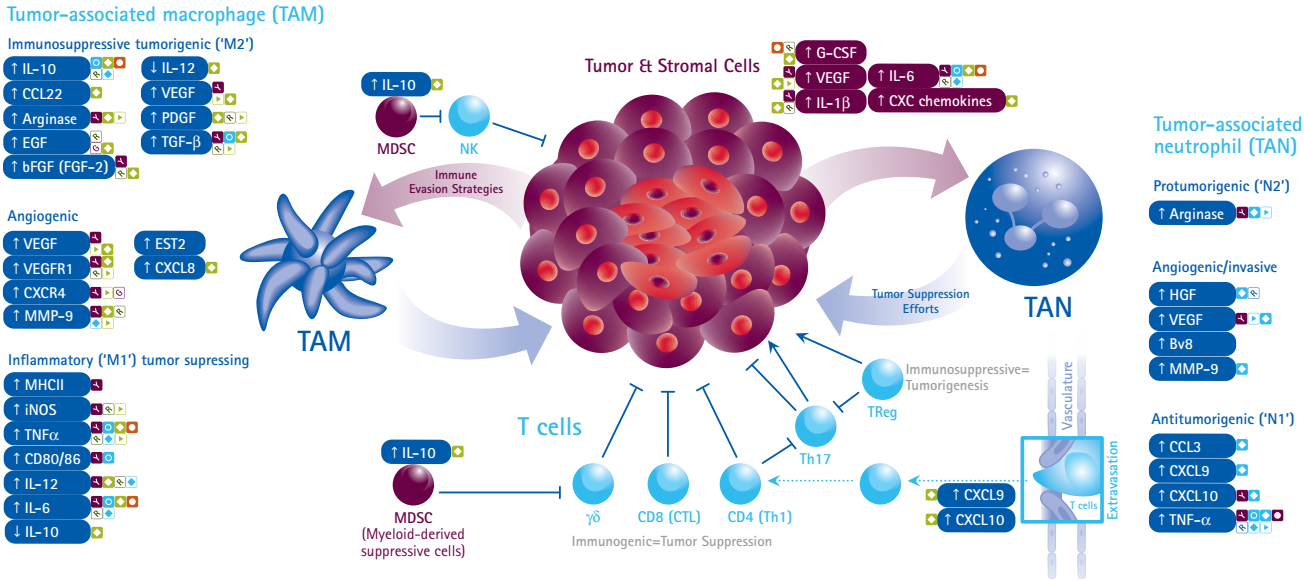
Cancer vaccines are one way to train the immune system to attack cancer cells. Usually, the vaccine consists of cells bearing antigens that are normally found on the tumor of interest, and these cells are often modified with adjuvants, which are chemical moieties that increase the immunogenicity of the antigen. Toll-like receptors, ligands, and bacterial cell surface components are examples of adjuvants. Adding cytokines, such as interferon or GM-CSF, activates the immune system further.

Immune checkpoint blockade therapies are another way to enable the patient's immune system to attack tumors. The first approved checkpoint blockade therapy targeted cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) in order to enable CTLs to attack tumors. Since then, other drugs have been developed that bind immune checkpoint proteins, such as PD-1, PD-L1, LAG-3, and CD47.

Finally, adoptive T-cell therapies, including chimeric antigen receptor-bearing (CAR) T cells, are a way to directly deliver immune cells that are programmed to attack tumor cells.

The latest clinical trials suggest that the best results may be achieved using immunotherapy combined with careful dosing of targeted chemotherapeutic, and taking into account the unique characteristics of each tumor's microenvironment.

The Tumor Microenvironment



PRODUCT SELECTION KEY

- Antibody
- Fluorophore-conjugated Antibody
- MILLIPLEX® Assays
- ELISPOT™ Assay
- ELISA Kit
- Recombinant Protein
- Inhibitor
- guava®/FlowCellect® Flow Cytometry Kit

Tumors escape immune control by modulating their microenvironment. The secrete immunosuppressive signals to tumor-associated macrophages, which in turn secrete tumor-suppressing proteins. Tumor-associated neutrophils carry out both tumorigenic and anti-tumorigenic functions. T cells and T helper cells act to suppress tumors, although immunosuppressive Treg cells can block T cell function.

Featured Publications

1. Maude SL et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014 Oct 16;371(16):1507-17.
2. Garon EB et al; KEYNOTE-001 Investigators. Pembrolizumab for the Treatment of Non-Small-Cell Lung Cancer. N Engl J Med. 2015 May 21;372(21):2018-28.
3. Le DT et al. Safety and Survival With GVAX Pancreas Prime and Listeria Monocytogenes-Expressing Mesothelin (CRS-207) Boost Vaccines for Metastatic Pancreatic Cancer. J Clin Oncol. 2015 Apr 20;33(12):1325-33.

Cancer Immunology

Featured Technique:

Flow Cytometry

Characterization of lymphocyte subtypes and cytokine signaling is essential for understanding the complex nature of the immune system. Activation by antigens, suppression of normal immune activation, and disease states can affect the phenotypes of lymphocytes. Flow cytometry is a statistically powerful technique for characterizing and/or sorting heterogeneous, suspended cell populations on the basis of physical characteristics and fluorescence. Flow cytometry relies on hydrodynamic focusing of a cell suspension sample to create a single-cell stream which passes in front of a laser. The manner in which the cell scatters incident light is used to determine the size and intracellular complexity of cells at a rate of thousands of particles per second. This analog data is then converted to digital data which can be quantified and plotted in two (or three) dimensions.

EMD Millipore's Milli-Mark® conjugated primary antibodies are fully optimized for fast, easy, and accurate multiparametric flow cytometry. These antibodies are versatile and highly stable, so you can run samples sequentially or as a large group, without compromising results. You can build your own assay with any compatible flow cytometry reagents and use automated compensation following acquisition.

Advantages of Milli-Mark® Conjugated Antibodies

- Most versatile and stable antibody format available
- Build your own assays for Immunophenotyping, Activation, and Immune Response
- Validated across multiple platforms

Technical Tip

Use nonenzymatic methods for detaching adherent cells from culture surfaces whenever possible to avoid unintentional cleaving of antigen from cell surfaces. When adherent cells are not amenable to detachment by nonenzymatic means, it is essential to use enzymes that are selective for attachment proteins (such as Accutase® enzyme, Catalog No. SCR005), rather than general proteases such as trypsin.

The Hallmark & Drug Development

Nivolumab, a monoclonal antibody targeting the PD-1 immune checkpoint protein, in combination with ipilimumab, which targets CTLA-4, was shown in clinical trials to elicit responses in over 60% of previously untreated metastatic melanoma patients.

Featured Cancer: Acute Lymphoblastic Leukemia (ALL)

Patients with relapsed ALL often have few therapeutic options. In a study treating 30 ALL patients with CAR T cells, 27 patients became cancer-free, a result which has generated much optimism regarding the potential of adoptive cell therapies.

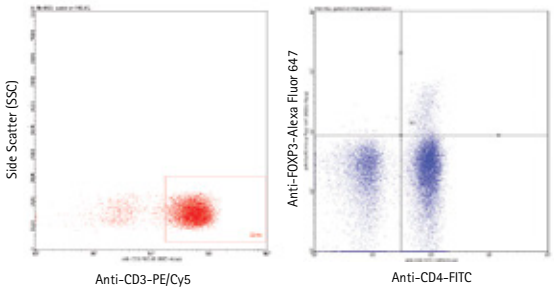
Featured Solution:

Characterization of Treg Cells Using Validated Conjugated Primary Antibodies

(Catalog No. FCIM025118)

Regulatory T-cells (Tregs) are a subpopulation of helper T-cells which are CD3+, CD4+, CD25+, and express the transcription factor FOXP3. Tregs 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.

Phenotypically distinguish and quantify human regulatory T-cells with high accuracy and specificity, using directly conjugated antibodies validated to work in flow cytometry. We offer conjugated antibodies for CD3, CD4, CD25 and FoxP3 (see table) as well as optimized reagents for fixation and permeabilization, which are critical steps for analyzing intracellular markers.



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.

Conjugated Antibodies

Description	Catalog No.
Milli-Mark® Anti-CD3 -PECy5 Antibody, clone UCHT1	FCMAB169C5
Milli-Mark® Anti-CD4 -FITC Antibody, clone MT310	FCMAB170F
Milli-Mark® Anti-CD25 Antibody (human), PE-Cy7, clone BC96	MABF547
Monoclonal Anti-FOXP3-PE antibody produced in mouse, clone 3G3 (order from **www.sigmaaldrich.com**)	SAB4700611

Fixation

Description	Catalog No.
Paraformaldehyde, reagent grade, crystalline (order from **www.sigmaaldrich.com**)	P6148

Permeabilization

Description	Catalog No.
Saponin, for molecular biology, used as non-ionic surfactant (order from **www.sigmaaldrich.com**)	47036

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Cancer Immunology:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-ABCB5, clone 5H3C6	MABC711
	Anti-Blys/TALL-1/BAFF/THANK	07-167
	Anti-C-X-C Chemokine Receptor 4, extracellular loop	AB1847
	Anti-C-X-C Chemokine Receptor 4, NT	AB1846
	Anti-CCL21, clone EPR6218, Rabbit Monoclonal	MABN1079
	Anti-CD133 (Prominin-1), clone 17A6.1	MAB4399
	Anti-CD81	ABC759
	Anti-CTLA4 (CD152), clone 9H10	04-963
	Anti-CXCL10	ABF50
	Anti-CXCR4, clone 12G5	MABF981
	Anti-Ido1, clone 8G11	MABS485
	Anti-IL-10 (human), PE, clone JES3-19F1	MABF328
	Anti-IL-12/IL-23 p40 (mouse), PE, clone C17.8	MABF1513
	Anti-IL-12R-b-1	06-1089
	Anti-IL27RA	ABF295
	Anti-Interleukin 10 Receptor a (IL-10RA)	06-1067
	Anti-Interleukin 12 (IL-12), clone 8.6	04-961
	Anti-Interleukin 21 (IL-21), clone 1G8	04-1583
	Anti-Interleukin-10	ABF13
	Anti-Interleukin-6	AB1421
	Anti-Interleukin-6 (IL-6), clone 1G3	MABF41
	Anti-JAK3, clone HL423	04-011
	Anti-LAG3 Antibody, clone C9B7W (Azide Free)	MABF846
	Anti-Macrophages/Granulocytes, clone OX-41	MAB1407P
	Anti-Osteoprotegerin/TNFRSF11B	ABC463
	Anti-TLR1, CT	06-006
	Anti-TLR2	06-1119
	Anti-TLR3, clone 2B11.2	MABF72
	Anti-TLR4, clone 13E1.1	MABF85
	Anti-TNF-α (human), PE, clone MAb11	MABF352
	Anti-TNF-α receptor-associated factor 1 (TRAF1), clone 11B2.2	MABC72
	Anti-TNF-α, clone 195	MAB1096

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Cancer Immunology:

Research Solutions	Description	Catalog No.
Related Antibodies (continued)	Anti-Toll-Interacting Protein (TOLLIP)	06-1109
	Anti-Traf2	ABC47
	Anti-Traf4, clone 5MLN-2H1	MABC985
	Anti-Traf6	06-1110
	Anti-Tumor Necrosis Factor-α(TNF-α)	AB1441
	Milli-Mark® Anti-Mouse IL-17 FITC, clone TC11-18H10.1	FCMAB255F
Assays and Kits	FlowCellect® Human FOXP3 Treg Characterization Kit	FCIM025118
	FlowCellect® Mouse FoxP3 Identification Kit	FCIM025126
	FlowCellect® Human Lymphocyte ZAP-70 Characterization Kit	FCIM025122
	FlowCellect® Mouse Th1/Th17 Intracellular Cytokine Kit	FCIM025138
	MILLIPLEX® MAP Human Circulating Cancer Biomarker Panel 1, magnetic	HCCBP1MAG-58K
Small Molecules	Dihydroorotate Dehydrogenase Inhibitor, Brequinar	508321
	FTY720	344597
	InhibitorSelect™ JAK/STAT Signaling Pathway Inhibitor Panel	420138
	InSolution™ JAK Inhibitor I	420097
	InSolution™ TLR4 Inhibitor, TAK-242	508336
	JAK 2 Inhibitor III, SD-1029	573098
	OXE-R-Gβγ Coupling Modulator	370681
	STAT 3 Inhibitor III, WP1066	573097
	STAT3 Inhibitor V, Stattic	573099
	TLR1/TLR2 Antagonist, CU-CPT22	614305
	TLR4 Inhibitor, TAK-242	614316
	TLR8 Agonist, cpd14b	530409
	Wogonin, *S. baicalensis*	681670

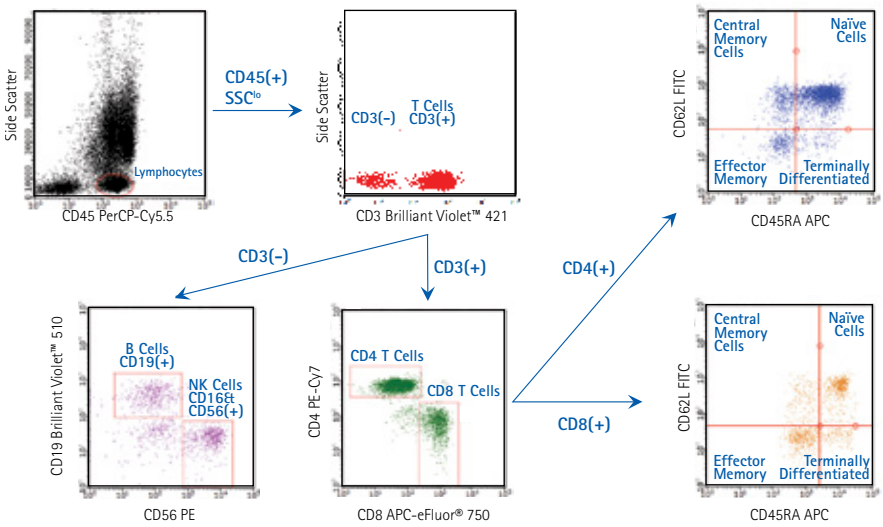
For a complete selection, visit: **www.emdmillipore.com**

TECHNOLOGY HIGHLIGHT

Multiparametric phenotyping of immune cell subpopulations using Guava® benchtop flow cytometers

Cellular health enumeration and the assessment of a heterogeneous cellular sample, such as whole blood or peripheral blood mononuclear cell (PBMCs) samples, often requires measuring many parameters to accurately characterize the subpopulations in combination with cellular health in a given sample. Identification and quantitation percentages of these subpopulations are often critical for defining the immune cell response as well as for understanding functional studies such as cytokine secretion, cellular proliferation and other processes. Traditional multicolor flow cytometers have posed challenges in multiparametric experiments like these because of difficulties in managing the fluorescent compensation necessary as well as the complexity in acquiring and analyzng these types of experiments. Typically, existing solutions are expensive, cumbersome to run and require extensive training or dedicated personnel.

In this study, we present data obtained from simplified methods that allow the identification of multiple subsets of cells, including T, B, natural killer (NK), naïve and memory cells, while simultaneously assessing the state of cell health using a novel instrument platform, the guava easyCyte™ 12 benchtop flow cytometry system. The guava easyCyte™ 12 flow cytometer is an ideal instrument for a wide range of experiments, including those that use an activation agent or study cytotoxic treatments as well as those that study the culture conditions needed to isolate and maintain a unique subset population.



Rapid subpopulation characterization in whole blood samples. Gating was done with a Side Scatter (SSC) vs. CD45 plot to gate on the CD45 (+) lymphocyte cells. Lymphocytes were gated into a SSC vs. CD3 plot from this CD3(+) and CD3(-) events were further analyzed. The CD3 (+) gate was used to identify the CD4+ and CD8+ positive cells T cells. CD4 and CD8 naïve memory cells were further discriminated by evaluating each population in a CD45RA (Naïve T cells) vs. CD62L (memory T cells). To separate the natural killer (NK) and B cells from the lymphocytes, CD3 (-) negative cells were gated into a plot comparing CD19 (B cells) and CD16+56 (NK cells).

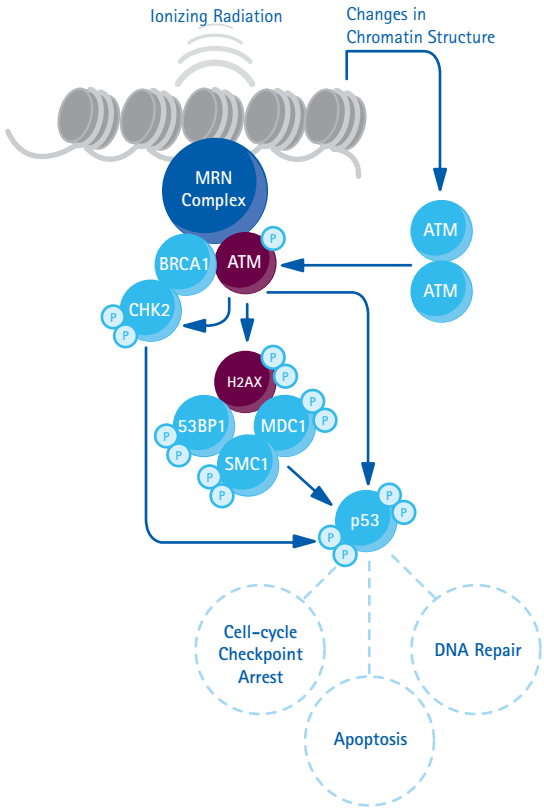
Genomic Instability and Mutation

This hallmark is one that can actually reveal the environmental causes of cancer.

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 or promote genomic integrity.

However, if any of these tumor suppressing mechanisms (such as p53) are disabled, the cell experiences increased mutations, chromosomal damage, recombination, and possibly aneuploidy.

With genome sequence data now available from thousands of tumors, hypermutation signatures (known as "kataegis") are now coming to light and revealing mechanisms of carcinogenesis, which includes effect of certain food additives and pollutants and pathogen infection and transposable elements.



Phospho H2A.X and phospho ATM are biomarkers of DNA damage. Flanking the sites of double-strand breaks in mammalian DNA, histone H2A.X is phosphorylated in what is thought to be a critical first step in the recruitment of repair factors to sites of DNA damage. ATM (ataxia telangiectasia mutated) is one of the earliest kinases recruited to DSB sites, and is the principal kinase responsible for phosphorylation of H2A.X at Ser139.

Featured Publications

1. Walker BA et al. APOBEC family mutational signatures are associated with poor prognosis translocations in multiple myeloma. Nat Commun. 2015 Apr 23;6:6997.
2. Okada N et al. A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. Genes Dev. 2014 Mar 1;28(5):438-50.
3. Kumar M, et al. Negative regulation of the tumor suppressor p53 gene by microRNAs. Oncogene. 2011 Feb 17;30(7):843-53.

Genomic Instability

Featured Technique:

Bead-based Multiplex Assays

Signaling molecules in the DNA damage pathway are coordinately regulated by phosphorylation, and understanding the role of this pathway requires the ability to simultaneously measure the phosphorylation status of multiple protein targets. Several assays to examine phosphorylation status are currently available, including Western blotting, ELISA, reverse phase arrays, quantitative cell imaging and mass spectrometry. Many of these assays are either limited to measuring only one analyte at a time, or are excessively difficult or expensive.

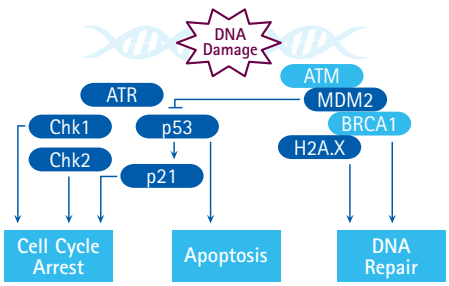
Bead-based multiplex assays, such as those using Luminex xMAP® technology, have enabled the high-throughput measurement of phosphorylation levels of multiple proteins simultaneously, which give the advantages of reduced sample volume, time and cost compared to traditional methods.

Featured Solution:

MILLIPLEX® MAP 7-plex Human DNA Damage/Genotoxicity Kit

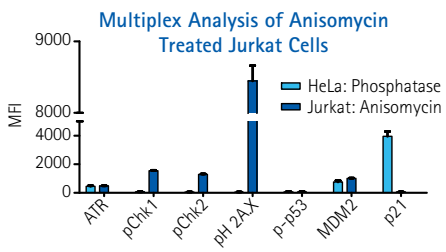
(Catalog No. 48-621MAG)

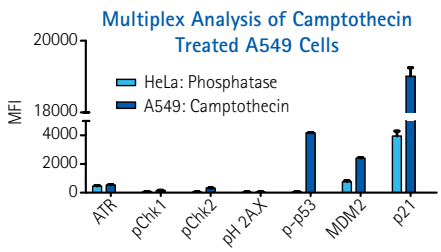
EMD Millipore's MILLIPLEX® MAP DNA Damage/Genotoxicity Kit is a magnetic bead-based immunoassay that simultaneously detects seven proteins in the DNA damage/genotoxicity pathway in a single sample, enabling the measurement of phosphorylation changes in this pathway. This kit is used to detect changes in phosphorylated Chk1 (Ser345), Chk2 (Thr68), H2A.X (Ser139), and p53 (Ser15) as well as total protein levels of ATR, MDM2, and p21 in cell lysates using the Luminex® system. The detection assay is a rapid, convenient alternative to Western Blotting and immunoprecipitation procedures. Each kit has sufficient reagents for one 96 well plate assay.



MILLIPLEX® MAP 7-plex DNA Damage/Genotoxicity Kit Analytes
ATR (Total)
Chk1 (Ser345)
Chk2 (Thr68)
H2A.X (Ser139)
MDM2 (Total)
p21 (Total)
p53 (Ser15)

Simplified schematic showing the DNA damage/genotoxicity pathway. Analytes detected using the MILLIPLEX® MAP 7-plex DNA Damage/Genotoxicity Kit are listed in the table above and highlighted in the schematic.





Multiplex analysis of Jurkat and A549 cells treated with anisomycin or camptothecin. HeLa cells treated with lambda phosphatase (negative control), Jurkat cells stimulated with 25 µM anisomycin (4 hours) and A549 cells stimulated with 5 µM camptothecin (overnight) 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 1 were analyzed according the Assay protocol (lysate incubation at 4°C overnight). The Median Fluorescence Intensity (MFI) was measured with the Luminex system. The figures represent the average and standard deviation of three replicate wells.

Technical Tip

When injecting a substance into the tail vein of a small animal (e.g., rat) it is best to submerge the tail in slightly warm water (37°C) for a minute and then wipe with ethanol or isopropyl alcohol. This will cause blood vessel to swell and become visible. Use a smaller gauge needle (e.g., 22-gauge needle) and inject with a slight pressure.

The Hallmark & Drug Development

MRX34, a mimic of miR-34, which positively regulates p53, is undergoing Phase I clinical trials in liver cancer patients. Preclinical studies showed that this drug was able to reduce tumor burden and decrease oncogene expression.

Featured Cancer: Ovarian Cancer

Severe genomic instability is a hallmark of BRCA-mutated ovarian cancers Olaparib, the first inhibitor of the DNA repair enzyme, poly ADP ribose polymerase (PARP), was approved by the U.S. FDA in 2014 for the treatment of this disease.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Genomic Instability:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-acetyl-p53 (Lys382), clone EPR358(2), rabbit monoclonal	04-1146
	Anti-ATM	07-1286
	Anti-ATM, clone Y170, rabbit monoclonal	04-200
	Anti-BRCA1	07-434
	Anti-BRCA1, clone BR64	MAB4132
	Anti-BRCA1, clone MS110	MABC199
	Anti-BRCA1, clone MS13	MABC161
	Anti-Chk2, clone 7	05-649
	Anti-MDM2 (Ab-1) Mouse mAb (IF2)	OP46
	Anti-MDM2, clone 2A10	MABE281
	Anti-MDM2, clone 3G9	04-1530
	Anti-Mdmx, clone 8C6	04-1555
	Anti-p53 (Ab-1) (Pantropic) Mouse mAb (PAb421)	OP03
	Anti-p53 (Ab-3) (Mutant) Mouse mAb (PAb240)	OP29L
	Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1)	OP43
	Anti-p53 (Ab-7) (Pantropic) Sheep pAb	PC35
	Anti-p53 (NT), clone Y5, rabbit monoclonal	04-1083
	Anti-p53 (pantropic), clone DO-1	MABE327
	Anti-p53 (wild type), clone PAb1620	MABE339
	Anti-p53, clone PAb421	MABE283
	Anti-phospho-ATM (Ser1981), clone 10H11.E12	05-740
	Anti-phospho-BRCA1 (Ser1423)	07-635
	Anti-phospho-Chk2 (Ser33/Ser35), clone EPR916(2)Y, rabbit monoclonal	04-1141
	Anti-phospho-Chk2 (Thr68)	ABE33
	Anti-phospho-p53 (Ser33), clone EP2393Y, rabbit monoclonal	MABE199
	Anti-Plk1	05-844
	Anti-PON1, clone 2H7	ABC155
	Anti-RIF1	ABC302
	Anti-RRM2	ABC106
	Anti-TRF1, clone BED5 57-6	04-638
	Anti-TRF2, clone 4A794	05-521
	ATM/Chk2 Pathway Explorer Antibody MiniPack	15-116
	ChIPAb+™ p53 - ChIP Validated Antibody and Primer Set	17-613

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Genomic Instability:

Research Solutions	Description	Catalog No.
Kits and Assays	H2A.X Phosphorylation Assay Kit, Flow Cytometry	17-344
	H2A.X Phosphorylation Assay Kit, Chemiluminescence Detection	17-327
	FlowCellect® Histone H2A.X Phosphorylation Assay Kit	FCCS100182
	FlowCellect® Cell Cycle Checkpoint H2A.X DNA Damage Kit	FCCH025142
	FlowCellect® DNA Damage Histone H2A.X Dual Detection Kit	FCCS025153
	MILLIPLEX® MAP Phospho-p53 (Ser15) MAPmate™ Assay	46-663
	MILLIPLEX® MAP Human Phospho-Histone H2A.X (Ser139) MAPmate™ Assay	46-692
	MILLIPLEX® MAP H2A.X (Ser139) Magnetic Bead MAPmate™ Assay	46-692MAG
	MILLIPLEX® MAP p53 (Ser15) Magnetic Bead MAPmate™ Assay	46-663MAG
	FragEL™ DNA Fragmentation Detection Kit, Colorimetric - Klenow Enzyme	QIA21
	FragEL™ DNA Fragmentation Detection Kit, Colorimetric - TdT Enzyme	QIA33
	FragEL™ DNA Fragmentation Detection Kit, Fluorescent - TdT Enzyme	QIA39
Small Molecules	ATM Kinase Inhibitor	118500
	ATM Kinase Inhibitor *KU-55933*	118500
	ATM/ATR Kinase Inhibitor	118501
	ATM/ATR Kinase Inhibitor *CGK773*	118501
	ATR/CDK Inhibitor, NU6027	189299
	Compound 401	234501
	DNA-PK Inhibitor	260960
	DNA-PK Inhibitor II	260961
	DNA-PK Inhibitor III	260962
	DNA-PK Inhibitor V	260964
	InSolution™ ATM Kinase Inhibitor	118502
	InSolution™ Nutlin-3, Racemic	444151
	MDM2 Antagonist, Nutlin-3, Racemic	444144
	MDM2 Inhibitor	444145
	MDMX Inhibitor, NSC207895	444158
	Mutant p53 Reactivator, RETRA	506164
	p53 Activator III, RITA	506149
	p53 Activator VII, STIMA-1	506168
	PARP Inhibitor Set	528820
	PARP Inhibitor VIII, PJ34	528150
	Pfithrin-a, p-Nitro, Cyclic	506154
	PRIMA-1	530050
	RAD51 Inhibitor, B02	553525
	TMEM16A Inhibitor, T16Ainh-A01	613551

For a complete selection, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

A quantitative approach for interrogation of H2A.X and ATM DNA damage signaling using the Muse® cell analyzer

Traditionally, DNA damage has been measured by Western blotting (WB) techniques using phospho-sensitive antibodies. WB requires the production of cell lysates, during which population heterogeneity is lost. Markers of DSB induction may vary on individual cells depending on factors such as cell cycle status, rendering WB and other methods that require homogenization of cell populations insensitive to characterization of individual cell status. Moreover, WB readouts are qualitative in that they do not provide the ability to evaluate cell damage responses with statistical power. In contrast, cytometric methods for interrogating DNA damage provide multiparameter data with single cell resolution.

The Muse® Cell Analyzer is a compact and highly intuitive benchtop instrument designed for 3-parameter analysis of fluorescent populations. The Muse® integrated computer and software package includes a Multi-Color DNA Damage module for rapid assessment of phosphorylated histone H2A.X and ATM on a cell-by-cell basis via capillary-based flow cytometry. To assess the capacity for the Muse® system to quantitatively assess downstream markers of DNA DSB, we evaluated phosphorylation of both H2A.X and ATM in cells treated with the topoisomerase inhibitor etoposide.

Analysis at the cellular level demonstrated the ability to reveal a time-dependent activation for each target as the exposure time to etoposide was prolonged. Similarly, ATM and H2A.X activation trends were evaluated in HeLa cells exposed to UV irradiation, and results established the capacity of the Muse® system to quantitatively measure DNA damage secondary to physical as well as chemical triggers.

Rapid analysis of DNA damage in HeLa cells with respect to etoposide time course. HeLa cells were exposed to 10 µM etoposide for 1, 2, 6, or 24 hours to induce DNA damage, and then stained with both anti-phospho-Histone H2A.X (Ser139) and anti-phospho-ATM (Ser1981) antibodies in multiplex. Samples were acquired using the Muse® Cell Analyzer and statistical results obtained using the Multi-color DNA Damage module of the built-in Muse® software.

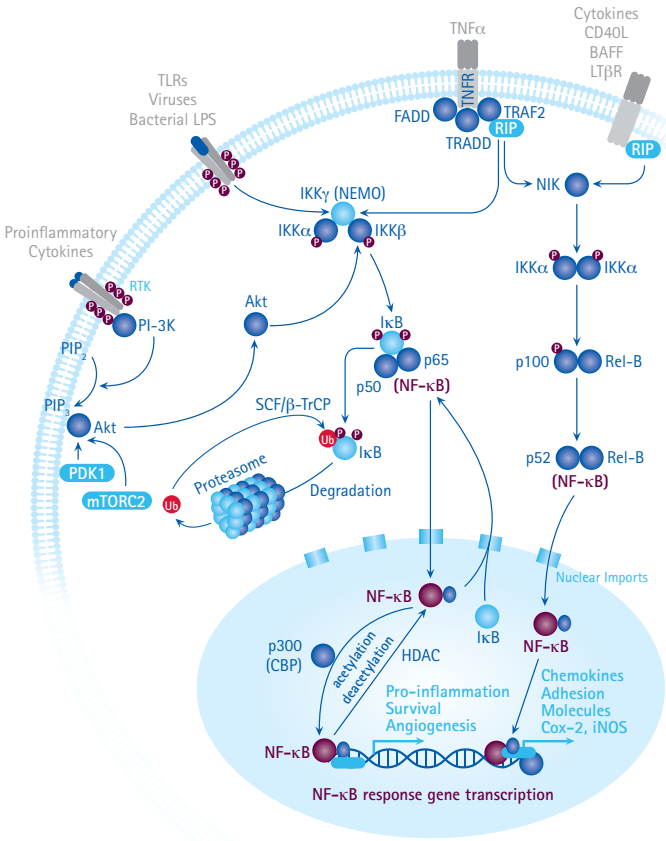
Notes

Tumor-promoting Inflammation

The good news? Inflammation might just make drug delivery easier.

In contrast to acute inflammation, such as that mediated by anti-tumorigenic Toll-like receptors, chronic inflammation mechanisms have been shown to foster tumorigenesis, as discussed in section 5 on angiogenesis. First, inflammation can increase vascular permeability via nitric oxide, free radicals, and 8-nitroguanosine. This tumor-specific permeability can enable selective delivery of anticancer drugs, particularly nanomedicines.

Also, 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.



NFκB integrates multiple signals, including chronic inflammation signals, to drive gene transcription in tumors. In addition to linking inflammation signals to tumorigenesis, NF-κB signaling exhibits crosstalk with immune checkpoint signaling, such as PD-1/PD-L1, in the tumor microenvironment.

Featured Publications

1. Williams TM, et al. The NLRP1 Inflammasome Attenuates Colitis and Colitis-Associated Tumorigenesis. J Immunol. 2015 Apr 1;194(7):3369-80.
2. Smith BR, et al. Selective uptake of single-walled carbon nanotubes by circulating monocytes for enhanced tumour delivery. Nat Nanotechnol. 2014 Jun;9(6):481-7.
3. Gowrishankar K, et al. Inducible but Not Constitutive Expression of PD-L1 in Human Melanoma Cells Is Dependent on Activation of NF-κB. PLoS One. 2015 Apr 6;10(4):e0123410.

Studying Inflammation

Featured Technique:

Transcription Factor Assays

At the end of most cell signaling pathways lies a change in gene transcription or post-transcriptional regulation that affects the level or localization of protein expression. Gene transcription is regulated by dynamic complexes of transcription factors, noncoding lincRNAs, coactivators and corepressors, histone acetylases, deacetylases, and other chromatin remodelers. Transcription factors are frequently the chief determinants of the composition and stability of these large transcription complexes, so it is important to develop robust assays to quantitate transcription factor activity.

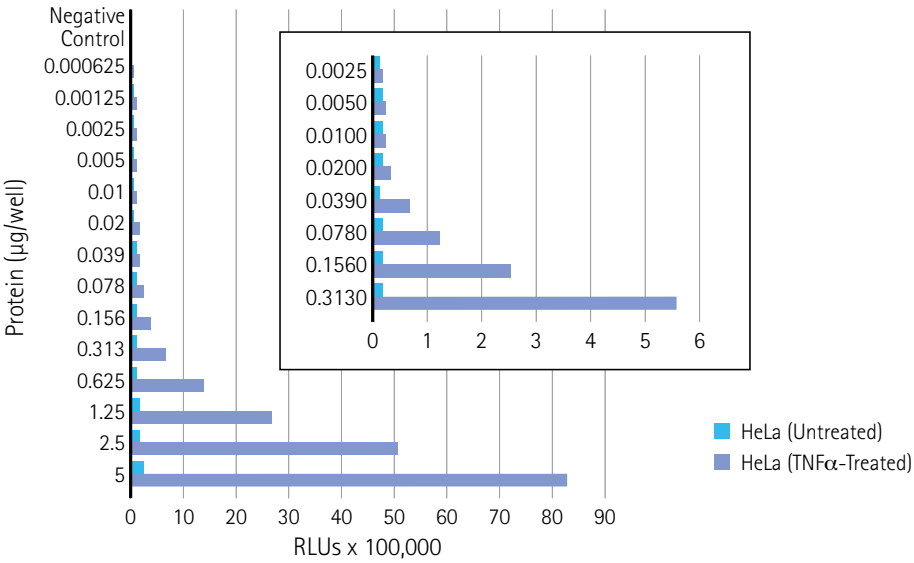
Analyze DNA-protein interactions without messy radioactivity or running time-consuming gels. EMD Millipore's EZ-TFA™ transcription factor assays provide a fast, sensitive method to detect specific DNA binding activity in whole cell or nuclear extracts. The assay enables high-throughput sample analysis with greater sensitivity than conventional electrophoretic mobility shift assays. Choose from universal kits that allow you to design an assay for your target of interest or one of our preconfigured target-specific assays.

Featured Solution:

NF-κB p65 EZ-TFA™ Transcription Factor Assay (Chemiluminescent)

(Catalog No. 70-620)

The transcription factor NF-κB (Nuclear Factor kappa B) is involved in the expression and regulation of a number of important cellular and physiological processes such as growth, development, apoptosis, immune and inflammatory responses. The p50/p65 heterodimer of NF-κB is the most abundant in cells. EMD Millipore's NF-κB EZ-TFA™ p50 and p65 assays are powerful tools for measuring active NF-κB in nuclear extracts.



The chemiluminescent NF-κB p65 transcription factor assay (Catalog No. 70-620) is extremely sensitive, with lower limits of detection in the ng of nuclear protein/well range. The assay's extreme dynamic range covers 5 logs of magnitude of detection as demonstrated here, using serial dilutions of untreated and TNFα-treated HeLa cell nuclear extracts from 0.000625 µg to 5 µg/well. Inset shows detail.

Technical Tip

Immunohistochemistry of tissue sections can be a powerful way to assess inflammation around tumors. Use a monoclonal antibody if concerned about specificity; a polyclonal antibody may be appropriate if expecting very low levels of antigen in the target tissue.

The Hallmark & Drug Development

Sulindac, an inhibitor of cyclooxygenase 2, has shown promising results in treatment of head and neck and colon cancers.

Featured Cancer: Colon Cancer

Multiple disease states that cause chronic inflammation in the bowel, such as inflammatory bowel disease and Crohn's disease, have been linked to colon cancer. In fact, patients with Crohn's disease or chronic ulcerative colitis are five to seven times more likely than unaffected individuals to develop colon cancer.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Inflammation:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-IKKb, clone Y466, rabbit monoclonal	04-366
	Anti-IKKα	07-1007
	Anti-IKKβ	07-1008
	Anti-IKKγ	05-631
	Anti-IKKε	07-580
	Anti-NF-κB p50 subunit	AB1602
	Anti-NF-κB p52	05-361
	Anti-NFκB p65, CT	06-418
	Anti-NFκB p65, CT	ABE347
	Anti-PD-L1	ABC324
	Anti-PD-L1, clone 29E.2A3.C6, Azide Free	MABC980
	Anti-PD-L2	ABC327
	Anti-PD-L2, clone 24F.10C12	MABC969
	Anti-PDCD1LG2	ABC221
	Anti-phospho JAK-2 (Tyr813)	09-242
	Anti-phospho-IkBα (Ser32/Ser36)	07-836
	Anti-phospho-NF-κB p65 (Ser536), clone EP2294Y, rabbit monoclonal	04-1000
	Milli-Mark® Anti-mouse CD44 (H-CAM)-PE, clone KM201	FCMAB426PE
	Milli-Mark® Anti-NF-κB p52-FITC	FCMAB346F
	PhosphoDetect™ Anti-STAT3 (pTyr705), clone 9E12	569384-1SET
Transcription Factor Assays	Universal EZ-TFA™, Colorimetric	70-500, 70-501
	Universal EZ-TFA™, Chemiluminescent	70-600, 70-601
	EZ-TFA™ NF-κB p50/p65, Colorimetric	70-510
	EZ-TFA™ NF-κB p50, Colorimetric	70-515
	EZ-TFA™ NF-κB p65, Colorimetric	70-520
	EZ-TFA™ NF-κB Family, Colorimetric	70-560
	EZ-TFA™ c-Jun, Colorimetric	70-540
	EZ-TFA™ c-Fos, Colorimetric	70-545
	EZ-TFA™ NF-κB p50/p65, Chemiluminescent	70-610
	EZ-TFA™ NF-κB p50, Chemiluminescent	70-615
	EZ-TFA™ NF-κB p65, Chemiluminescent	70-620
	EZ-TFA™ NF-κB Family, Chemiluminescent	70-660
	EZ-TFA™ c-Jun, Chemiluminescent	70-640
	EZ-TFA™ c-Fos, Chemiluminescent	70-645

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Inflammation:

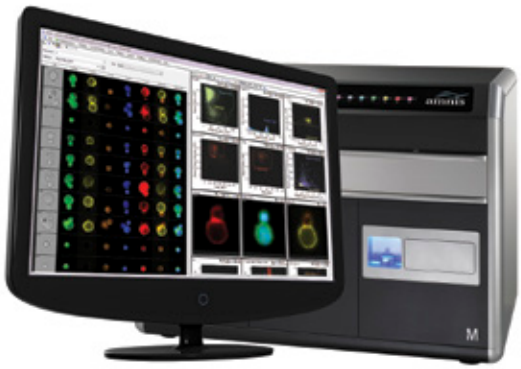
Research Solutions	Description	Catalog No.
Related Kits	Mouse IL-6 ELISA Kit	EZMIL6
	MILLIPLEX® MAP Total STAT1 MAPmate™	46-654
	MILLIPLEX® MAP TGFβ Signaling Pathway Magnetic Bead 6-Plex	48-614MAG
Small Molecules	AP-1/NF-κB Dual Inhibitor, SP100030	531535
	Calbiochem® InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel	481487
	Celastrol, Celastrus scandens	219465
	COX-2 Inhibitor II	236012
	COX-2 Inhibitor V, FK3311	236015
	Cyclooxygenase Inhibitor Set	239783
	IKK Inhibitor II, Wedelolactone	401474
	IKK Inhibitor III, BMS-345541	401480
	IKK-2 Inhibitor VIII	401487
	IKK-2 Inhibitor, SC-514	401479
	IKK-3 Inhibitor IX	401488
	InSolution NF-κB Activation Inhibitor	481407
	Meloxicam	444800
	NEMO-Binding Domain Binding Peptide, Negative Control	480030
	NEMO-Binding Domain Peptide, Cell-Perm.	480025
	NF-κB Activation Inhibitor	481406
	NF-κB Activation Inhibitor II, JSH-23	481408
	NF-κB Activation Inhibitor VII, CID-2858522	480457
	NF-κB Inhibitor Methysticin	480458
	NF-κB SN50, Cell-Permeable Inhibitor Peptide	481480

For a complete selection, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

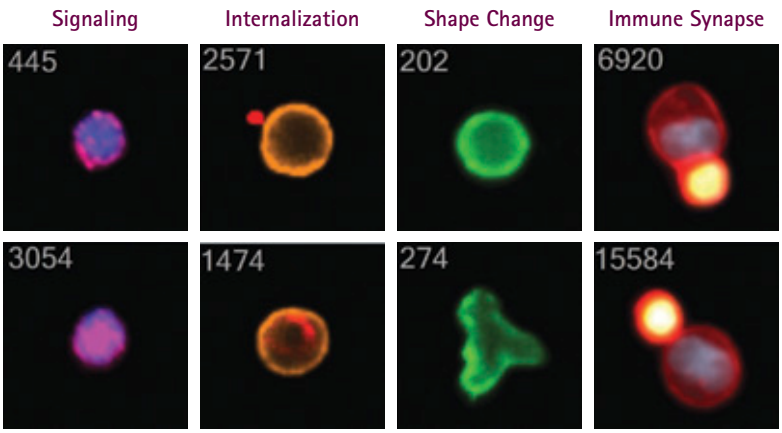
Accelerating inflammation/immune signaling research using imaging flow cytometry.

Imaging flow cytometry changes the way immunologists view data—literally.



Many assays for immune function require imaging, but immune cells present significant challenges to image-based analysis due to their rarity and the need for simultaneous multispectral immunophenotyping, making statistically robust quantitation difficult. Thus, immune function assays are ideally performed by the ImageStream®^{X} imaging flow cytometry platform, which quantifies imagery of large populations of cells. Our application note shows several examples of ImageStream®^{X} immune function assays, including activation of NF-κB translocation in whole blood cell plasmacytoid dendritic cells (pDC), internalization and trafficking of viral DNA within pDC, chemokine-induced monocyte shape change, and T cell-APC immune synapse formation. The results demonstrate the unique suitability of the ImageStream®^{X} system for immune function assays.

Read the entire application note "**Measurement of Immune Cell Function Using ImageStream®^{X} Cytometry**".



Imaging flow cytometry was used to measure NFκB translocation as well as other immune cell processes, such as internalization, shape change, and immune synapse.

Amnis® Imaging Flow Cytometers

Though statistically powerful, traditional non-imaging flow cytometry must often be augmented by microscopy for the confidence in signal localization and morphology image data can provide. However effective, microscopy continues to be generally low throughput, time-consuming, and subjective with respect to analysis.

Amnis® imaging flow cytometers (IFC) are the first instruments to integrate microscopy into flow, combining the advantages of visual data with unparalleled sensitivity in cytometry.

Both the FlowSight® and ImageStream®^{X} systems use a charge-coupled device (CCD) camera that enhances sensitivity to fluorescent signal while providing multicolor images of every cell or other event collected in flow.

Population characteristics and statistical data from thousands of cells per sample are complemented by visual data collected automatically for each event. Amnis® systems use advanced algorithms enabling analysis of cells or other events collected in flow that allows unprecedented characterization of populations using elements of visual data.

For more information on immunology research and other applications using Amnis® imaging flow cytometry, visit our website at: **www.emdmillipore.com/amnis**

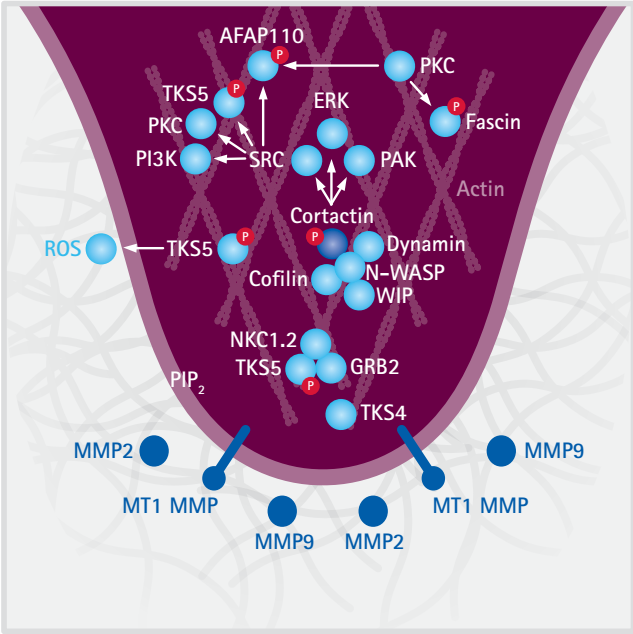
Notes

Tissue Invasion and Metastasis

Extracellular vesicles may be carving out the metastatic niche.

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, and involve cell-cell signaling via extracellular vesicles (carrying protein and ncRNA cargo) and ephrin-A1 binding.



Mechanisms of tissue invasion by invadopodia. Invadopodia are structures formed by metastatic tumor cells, which degrade the extracellular matrix via secreted matrix metalloproteases (MMPs). MMP secretion is thought to be regulated by TKS adaptor proteins, which also direct cell structure proteins to assemble the invadopodia.

Featured Publications

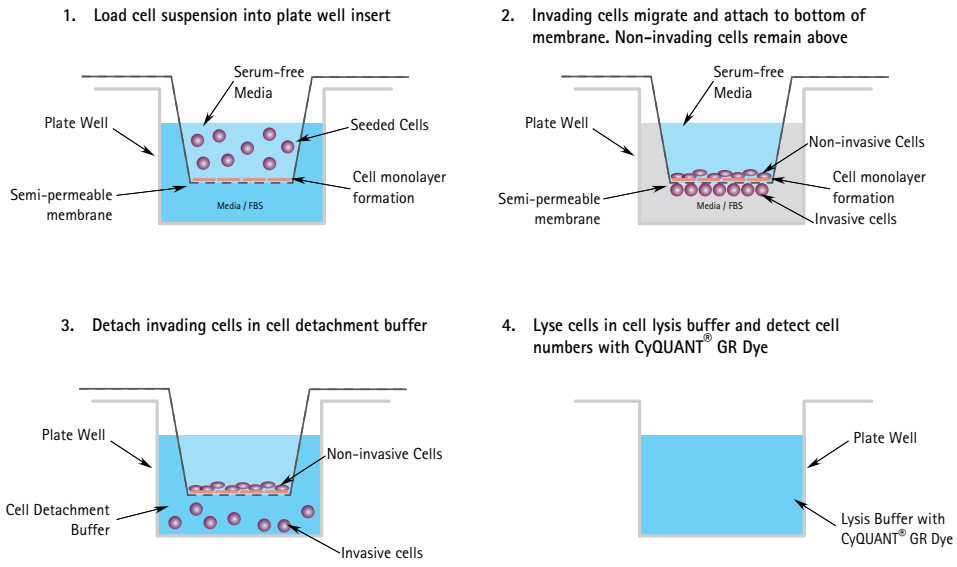
1. Reticker-Flynn NE, Bhatia SN. Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. Cancer Discov. 2015 Feb;5(2):168-81.
2. Otto B, et al. Molecular changes in pre-metastatic lymph nodes of esophageal cancer patients. PLoS One. 2014 Jul 21;9(7):e102552.
3. Ieguchi K, et al. ADAM12-cleaved ephrin-A1 contributes to lung metastasis. Oncogene. 2014 Apr 24;33(17):2179-90.

Invasion and Metastasis

Featured Technique:

Boyden Chamber

Microporous membrane inserts are widely used for cell migration and invasion assays. The most widely accepted of which is the Boyden chamber assay. The classic Boyden chamber system uses a hollow plastic chamber, sealed at one end with a porous membrane. This chamber is suspended over a larger well which may contain medium and/or chemoattractants. Cells are placed inside the chamber and allowed to migrate through the pores, to the other side of the membrane. Migratory cells are then stained and counted. Two forms of cell migration that can be studied using Boyden chambers are chemotaxis and haptotaxis.



Boyden Chamber assay.

In a standard Boyden assay, the pore diameter of the membrane is typically 3 to 12 µm, and is selected to suit the subject cells. Smaller pore size results in a greater challenge for the migrating cell. Most cells range in size from 30-50 µm and can migrate efficiently through 3-12 µm pores, whereas, lymphocytes (10 µm) can migrate through pores as small as 0.3 µm.

How to select the appropriate pore size for your cells:

- 3 µm pore size is appropriate for leukocyte or lymphocyte migration.
- 5 µm pore size is appropriate for a subset of fibroblast cells or cancer cells such as NIH-3T3 and MDA-MAB 231 cells. Also suitable for monocytes and macrophages.
- 8 µm pore size is appropriate for most cell types. This pore size supports optimal migration for most epithelial and fibroblast cells. Note - the 8 µm pore size is not appropriate for lymphocyte migration experiments.

EMD Millipore's QCM™ Boyden chamber cell invasion assays enable convenient and sensitive quantification of *in vitro* cell invasion through a basement membrane model, where a layer of ECM solution occludes the membrane pores, blocking non-invasive cells from migrating through it.

Technical Tip

Although wound healing, or "scratch", assays may appear to be simple ways to measure the migratory potential of cells, it may be difficult to generate reproducible scratches. Use a Cell Comb™ to generate multiple scratches at once; this simple tool may even permit biochemical analysis of migrating cells.

The Hallmark & Drug Development

Trabedersen, an antisense oligonucleotide drug that blocks the production of TGFβ2, has shown some success in treating metastases of pancreatic and brain tumors.

Featured Cancer: Breast Cancer

Key discoveries surrounding the mechanism by which tumor cells defined their metastatic niche were made in breast cancer, by the laboratories of Robert Weinberg, Joan Massagué, and others. Cancer cells, including cancer stem cells, cooperate with stromal cells to create the niche, sometimes after the primary tumor appears to have been eliminated.

Featured Solution:

QCM™ 96-well (8 µm) Cell Invasion Fluorometric Assay with ECMatrix™ membrane layer

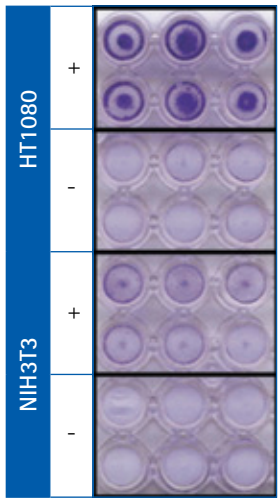
(Catalog No. ECM555)

EMD Millipore's QCM™ 96-well Invasion Assay provides a quick and efficient system for quantitative determination of various factors on cell invasion, including screening of pharmacological agents, evaluation of MMPs or other proteases responsible for cell invasion, or analysis of gene function in transfected cells.

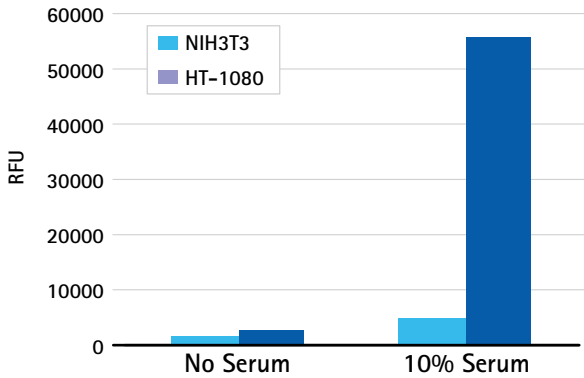
This kit provides an efficient system for evaluating the invasion of tumor cells through a basement membrane model. It contains a 96 well invasion plate based on the Boyden chamber principle. The plate contains 96 inserts, each with an 8 µm pore size polycarbonate membrane coated with a thin layer of ECMatrix™ (a reconstituted basement membrane matrix of proteins derived from the Engelbreth Holm-Swarm (EHS) mouse tumor). Human fibrosarcoma (HT-1080) and non-invasive fibroblasts (NIH3T3) have been used to test this kit.

The QCM™ 96-well Invasion Assay does not require cell labeling, scraping, washing or counting. The 96-well insert and homogenous fluorescence detection format allows for large-scale screening and quantitation.

A.



B.



Human Fibrosarcoma HT-1080 cells or non-invasive NIH3T3 cells were allowed to invade toward 10% FBS for 12 hrs. 100,000 cells were used in each assay. A: Invaded cells on the under side of the membrane were stained with 0.1% Crystal violet solution. B: Fluorescence measurements of dye demonstrates invasion of HT-1080 cells in the presence of FBS.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Invasion and Metastasis:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-Integrin αVβ3, clone LM609, azide-free	MAB1976Z
	Anti-B-Raf	07-453
	Anti-B-Raf, clone 4B3.2	05-1094
	Anti-erbB-2/HER-2	06-562
	Anti-phospho-erbB-2/HER-2 (Tyr1248)	06-229
	Anti-phospho-erbB-2/HER-2 (Tyr1248), Alexa Fluor® 555	16-236
	MMP/TIMP Family Antibody Sampler Kit I	ASK10
	Anti-MT1-MMP, hemopexin domain	AB6070
	Anti-MT1-MMP, catalytic domain	AB6005
	Anti-MMP-9, Hinge region	AB6008
	Anti-MMP-10	ABT289
	Anti-MMP-15	AB855
	Anti-MMP-16, clone 117-10C6	MAB3314
	Anti-MMP-9, catalytic domain	AB19016
	Anti-Met (extracellular), clone 4F8.2	05-1049
Related Proteins	Met (D1246N), active	14-818
	TIMP-2, human, recombinant	PF021
Kits and Assays	QCM™ Cell Invasion Assay with ECMatrix™ Membrane Layer, 24-well (8 µm), Colorimetric	ECM550
	QCM™ Collagen Cell Invasion Assay, 24-well (8 µm), Fluorometric	ECM552
	QCM™ Collagen Cell Invasion Assay, 96-well (8 µm), Fluorometric	ECM556
	QCM™ Tumor Cell Transendothelial Migration Assay (Colorimetric, 24 Assays)	ECM558
	QCM™ Tumor Cell Transendothelial Migration Assay (Fluorometric, 24 Assays)	ECM560
	QCM™ Gelatin Invadopodia Assay (Green)	ECM670
	QCM™ Gelatin Invadopodia Assay (Red)	ECM671
	QCM™ Endothelial Cell Invasion Assay (24 well, Colorimetric)	ECM210
	QCM™ Endothelial Cell Invasion Assay (24 well, Fluorometric)	ECM211
	Endothelial Cell Adhesion Assay	ECM645
	Integrin-mediated & ECM Cell Adhesion Arrays	ECM530
	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
	Millicell® µ-Migration Assay Kit	MMA205

For a complete selection, visit: www.emdmillipore.com

EMD Millipore offers effective solutions for research on topics related to Invasion and Metastasis:

Research Solutions	Description	Catalog No.
Small Molecules	(+)-Brefeldin A, *E. brefeldianum*	203729
	Actin Polymerization Interfering Agents Set	104850
	Arp2/3 Complex Inhibitor I, CK-666	182515
	Arp2/3 Complex Inhibitor II, CK-869	182516
	CA-074	205530
	CA-074 Me	205531
	Cathepsin B inhibitor I (Z-FA-FMK)	342000
	Cathepsin B Inhibitor II (Ac-LVK-CHO)	219385
	Cathepsin K Inhibitor III	219381
	Cathepsin L Inhibitor CAA0225	219502
	Cathepsin L Inhibitor III	219427
	Colchicine, *Colchicum autumnale*	234115
	Cytochalasin B, *H. dematioideum*	250233
	Cytochalasin D, *Zygosporium mansonii*	250255
	GM6001	CC1100
	GM6001 (MMP inhibitor)	364206
	Ilimaquinone	401250
	InSolution™ Latrunculin A, *L. magnifica*	428026
	InSolution™ Nocodazole	487929
	InSolution™ Paclitaxel, *Taxus* sp.	508227
	Integrin α M β 2 Ligand	407271
	Jasplakinolide, *Jaspis johnstoni*	420107
	Marimastat	444289
	MMP Inhibitor Set I	444255
	MMP-2 Inhibitor IV	444294
	MMP-2/MMP-9 Inhibitor I	444241
	MMP-2/MMP-9 Inhibitor V	444285
	MMP-9 Inhibitor I	444278
	N-WASP Inhibitor, 187-1	681660
	Phalloidin, *Amanita phalloides*	516640
	TGFβ RI Inhibitor II	616452
	Troponin Activator, CK-2017357	531230
	Wiskostatin	681525

For a complete selection, visit: www.emdmillipore.com

TECHNOLOGY HIGHLIGHT

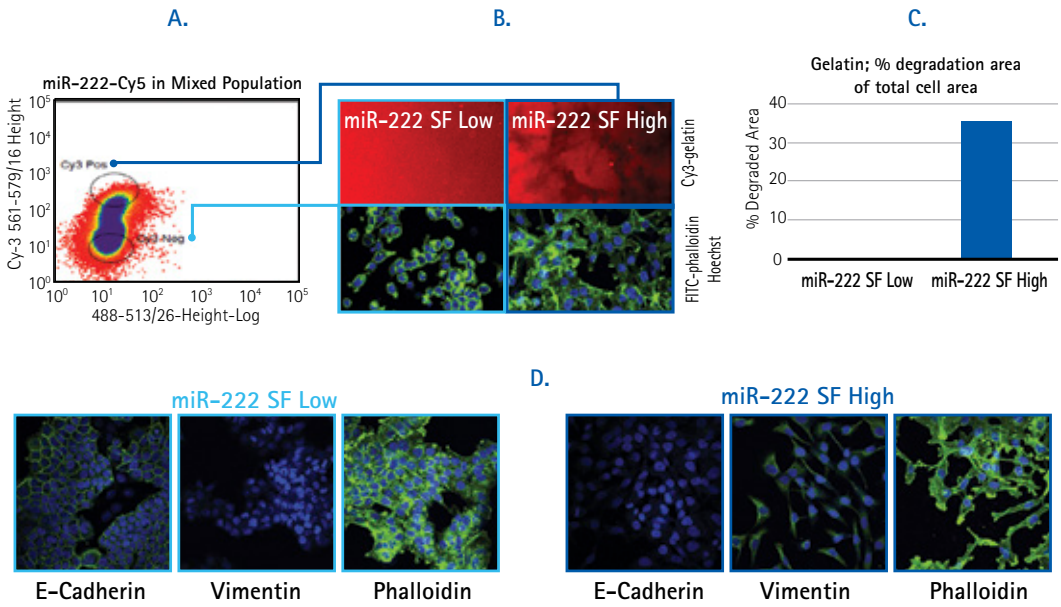
Sorting live cells based on miRNA expression and correlation with metastatic phenotypes

Cancer biomarkers have been used to identify transformed cells within heterogeneous tissues and cell lysates but currently only provide retrospective confirmation of disease state. Identifying RNA biomarkers in live cells provides the unique opportunity to understand the impact of tumor heterogeneity on the behavior of transformed cells and to enrich for cells based on RNA markers using fluorescent activated cell sorting.

Live cell sorting has traditionally been accomplished by detecting the presence of cell surface proteins using fluorescently labeled antibodies. However, the cell recovery rate is poor after irreversible antibody labeling. Furthermore, live cells cannot be sorted based on endogenous intracellular protein markers, because of the need for fixation.

Using novel SmartFlare™ RNA detection reagents, which are capable of detecting levels of RNA inside living cells, we have demonstrated the ability to sort and further propagate live cell populations purely based on gene expression levels or in combination with surface markers detected with antibodies. This technology eliminates the need for permeabilization or transfection reagents to interrogate the cytoplasmic content of cells, leaving the cells intact and viable after sorting. More importantly, because the particles are inert and leave the cells unharmed, the cells are available for use in downstream assays, enabling the measurement of additional biomarkers or collection of functional data.

We sorted breast cancer cells on the basis of RNA biomarkers. We sorted cells based on expression of miR-222 and further characterized the sort products with respect to expression of epithelial-mesenchymal transition (EMT) markers and formation of invadopodia.



"miR-222-high"and "miR-222-low" cells were sorted using Cy3 SmartFlare™ RNA probes specific to miR-222 (A) and analyzed using an invadopodia formation assay. Invadopodia that degraded the gelatin matrix could be seen as dark areas on the red gelatin substrate and quantified using ImageJ software (B and C). ICC using the antibodies listed was used for EMT characterization of the sort products (D).

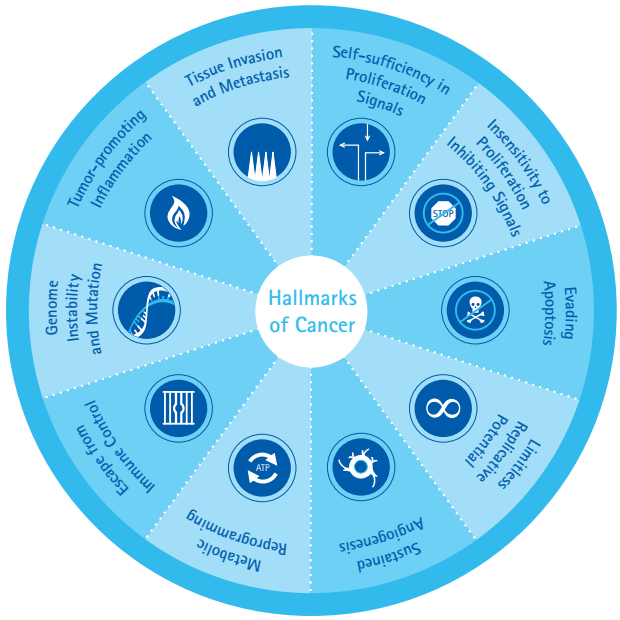
Select a SmartFlare™ live cell RNA detection probe from our wide selection or design your own at:
www.emdmillipore.com/smartflare

Notes

Notes

Hallmarks of Cancer

Research Solutions Guide



What's inside...

Ten sections, one for each of the Hallmarks, with:

- An introduction to the hallmark
- Informative illustrations
- Featured publications related to the hallmark
- Featured technique to study hallmark
- Featured solution using the technique
- *Technical Tip* (for research)
- *The Hallmark & Drug Development*
- *Featured Cancer* (informational bits)
- Effective solutions to advance your hallmark-related research
- Technology highlights

To place an order or receive technical assistance

In the U.S. and Canada, call toll-free
1-800-645-5476

For other countries across Europe and the world, please visit: **www.emdmillipore.com/offices**

For Technical Service, please visit:
www.emdmillipore.com/techservice

Get connected!

Join EMD Millipore on your favorite social media outlet for the latest updates, news, products, innovations, and contests!

facebook.com/EMDMillipore

twitter.com/EMD_Millipore

EMD MILLIPORE

www.emdmillipore.com/cancer

Lit No. PB5867EN00 BS-GEN-15-11632 01/2016