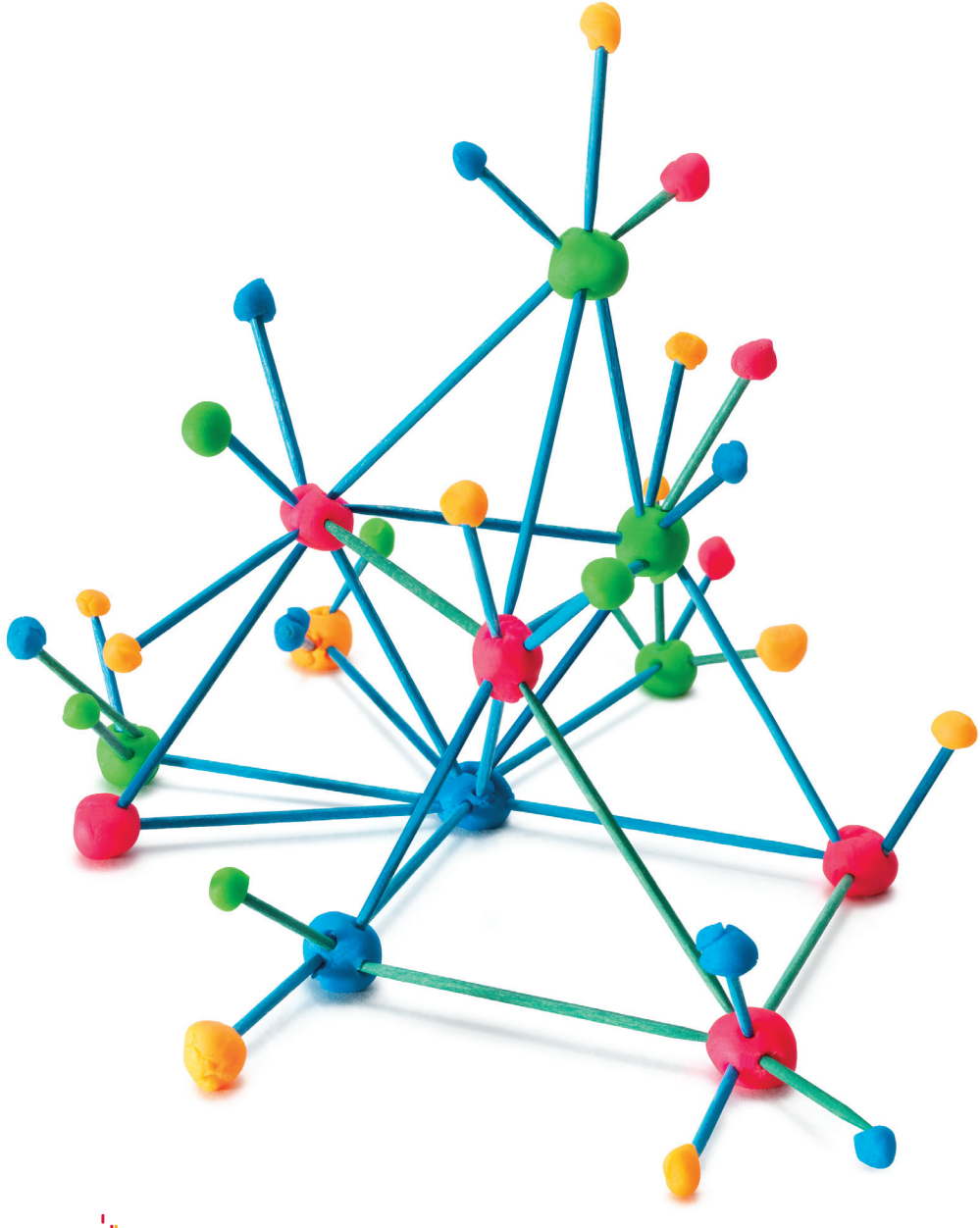
MERCK MILLIPORE

Product Selection Guide

Activate Your Discovery Network.

Antibodies, small molecule inhibitors, kits, assays and proteins for signaling research.



Merck Millipore is a Division of MERCK

Platforms, Technologies, and Services

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

CALBIOCHEM® SMALL MOLECULES

Small-molecule compounds, including inhibitors, activators, and other pathway modulators, are critical tools for researchers studying cell signaling. Chemical genetics, in which loss of function is imposed using small molecules, can reveal connections within signaling networks. Merck Millipore's Calbiochem® reagents have been cited in thousands of peer-reviewed publications. From libraries and pathway panels to individual reagents, the Calbiochem® line of products offers the widest and most cited selection of inhibitors and activators worldwide.

ANTIBODIES AND IMMUNOASSAYS

With the expertise of Upstate® and Chemicon®, Merck Millipore provides an extensive, focused, validated portfolio of antibodies and immunoassays, with breadth and depth in major research areas backed by excellent service and support. Merck Millipore also offers a variety of ELISAs in major research areas, including cell signaling, and novel tools for improving the Western blotting workflow.

CELL-BASED ASSAYS

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

FLOW CYTOMETRY ASSAYS AND SYSTEMS

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

MILLIPLEX® MAP MULTIPLEX ASSAYS

MILLIPLEX® MAP assays offer the broadest selection of multiplex kits and reagents in a wide variety of research areas, measuring multiple biomarkers using a small sample size. MILLIPLEX® MAP enables the simultaneous detection of multiple soluble or intracellular biomarkers. Using the Luminex® xMAP® bead-based technology, these flexible and customizable assays are exhaustively tested and qualified for sensitivity, specificity, reproducibility and wide dynamic range. Providing absolute, site-specific quantitation of phosphorylation, the MILLIPLEX® MAP EpiQuant™ assays are the most advanced platform for cell signaling analysis.

MOLECULAR BIOLOGY AND PROTEIN PREPARATION

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

CELLS AND CELL CULTURE

Merck Millipore's innovative cell culture solutions help optimize cell growth and maintenance for signaling research. We offer an extensive range of human and rodent stem cells, primary cells and media designed for most types of stem cells, including embryonic, mesenchymal, and neural stem cells. Our flexible sterile filtration devices offer fast flow and have many membrane options. Also available are microfluidic systems and cultureware to mimic *in vivo* conditions and provide coculture options.

Introduction

Cell signaling, often called signal transduction, is a complex network of receptors, enzymes, and messengers that enables cells to perceive, communicate with and respond to their environment. Cell signaling controls and regulates every aspect of cell function, including cell division, cell proliferation, and cell death.

Cell signaling may occur in three forms:

- **Extracellular** signals, such as contact with other cells, hormones, growth factors, chemoattractants, metal ions, or contact with extracellular matrix (ECM) components.
- Interaction between **cytosolic** signaling proteins in response to either intracellular molecules or messengers generated as a result of ligand binding to transmembrane receptors.
- **Nuclear** response to stimuli, ultimately manifested as changes in gene expression.

Despite the intricate and complex nature of signaling networks, the tools used to study them are based on the classic principles of protein detection and manipulation. Immunodetection has undergone technological advances, such as multiplex protein detection and multiparametric cell analysis. Nevertheless, the success of cell signaling research still largely depends on the quality, sensitivity, and specificity of the antibodies used for detection and small molecules to induce or block modifications in enzyme activities.

Combining its long-trusted immunodetection tools and reagents with the expertise of Calbiochem®, Upstate®, Chemicon® and Linco®, Merck Millipore is the leading partner for scientists in both basic cell signaling research as well as signaling pathway profiling for the drug discovery industry. By using this product guide in conjunction with our online resource (**www.millipore.com/signaling**), let us help you design an experimental strategy for understanding the signaling networks in your biological model of interest.

Table of Contents

Extracellular Signaling

The binding of molecules, such as hormones, growth factors, neurotransmitters, and pharmacological agents, to the extracellular domains of transmembrane receptors triggers many important signaling events inside the cell. Transmembrane receptors are some of the most abundant proteins in plasma membranes. These receptors bind agonists, which elicit a highly specific and selective response, and antagonists, which induce non-responsiveness by blocking a receptor. Understanding the biological activity associated with receptor-ligand interactions is central to unraveling signaling pathways and thus forms the framework for research, drug discovery and development programs.

Growth Factor Signaling

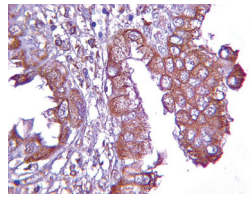
Growth factors are peptides that are secreted by a variety of cells, act through cell surface receptors and can elicit similar as well as distinct biological responses in their target cells. Growth factors can regulate cell growth, proliferation, differentiation and maturation, making growth factor signaling ideal for targeting in research, discovery and clinical environments. Major families of growth factor receptors are those with tyrosine kinase activity, G-protein coupled receptors and those with serine/threonine kinase activity. Typically, binding of growth factors to receptors on quiescent cells leads to the activation of intrinsic receptor-associated tyrosine kinase activity and concomitant phosphorylation of tyrosine residues of cytoplasmic proteins.

Featured Products

Anti-VEGF

(Catalogue No. ABS82)

VEGF (Vascular endothelial growth factor, VEGFA, VEGF-A), a dimeric ligand, is a highly specific mitogen for vascular endothelial cells. The expression of VEGF is potentiated in tumors in response to hypoxia. This process is aided by a variety of activated oncogenes and cytokines. VEGF plays a central role in the regulation of vasculogenesis and angiogenesis by inducing endothelial cell proliferation, migration, and maturation and by inhibiting their apoptosis.

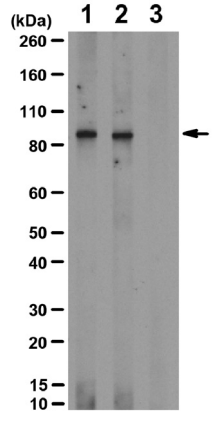


VEGF secretion detected in human placental villi using immunohistochemistry with Anti-VEGF (1:400, Cat. No. ABS82) and the Merck Millipore IHC Select® Detection Kit (Cat. No. DAB050).

Anti-phospho-IGF-1R (Tyr1161/Tyr1165/Tyr1166)

(Catalogue No. ABE332)

Insulin-like growth factor 1 receptor (IGF-1 receptor, IGF-1R) binds IGF-1, a peptide hormone secreted in response to growth hormone signaling. IGF-1R, a receptor tyrosine kinase, phosphorylates cytoplasmic signaling proteins, including Akt/mTOR pathway proteins, to promote growth and cell survival. IGF-1R is highly expressed in all cell types and tissues and is highly overexpressed in most malignant tissues.

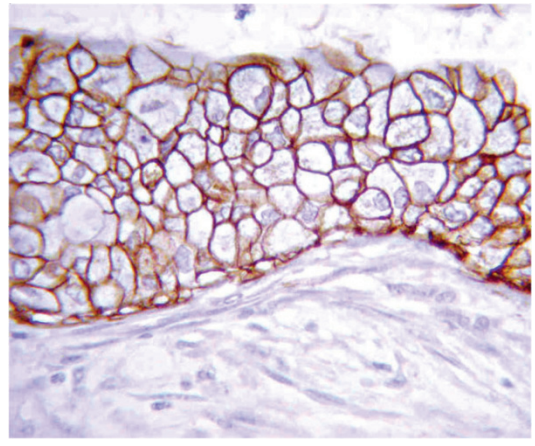


Peptide inhibition assay demonstrating that Anti-phospho-IGF-1R (1:200, Cat. No. ABE332) pre-incubated with specific phosphopeptide blocks detection of IGF-1R in IGF-1-treated HEK293 lysates (lane 3), whereas no peptide or incubation with non-phosphorylated peptide does not block detection of IGF-1R (lanes 1 and 2, respectively).

Anti-EGF

(Catalogue No. 07-1432)

Epidermal growth factor (EGF) has a profound effect on the differentiation of specific cells *in vivo* and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin. EGF binds to the EGF receptor (EGFR), which leads to cell proliferation, migration, adhesion and other processes via the MAPK, JNK, Akt and other pathways.

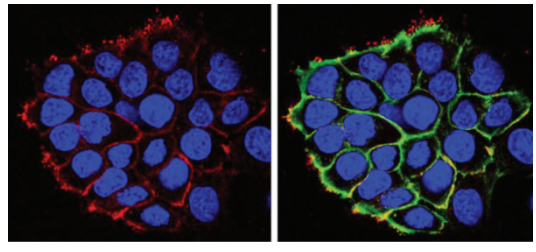


EGF staining of colon cancer tissue using Anti-EGF (1:100, Cat. No. 07-1432) and the Merck Millipore IHC Select® HRP-DAB detection system (Cat. No. DAB500).

Anti-EGFR

(Catalogue No. 05-1047)

The EGF receptor (EGFR) is the cell-surface receptor for members of the EGF family and is activated by binding specific ligands, including the transforming growth factor α (TGFα). Upon activation, EGFR homodimerizes, stimulating its intracellular protein tyrosine kinase activity, leading to cell proliferation. Certain EGFR mutations are associated with different forms of cancer.

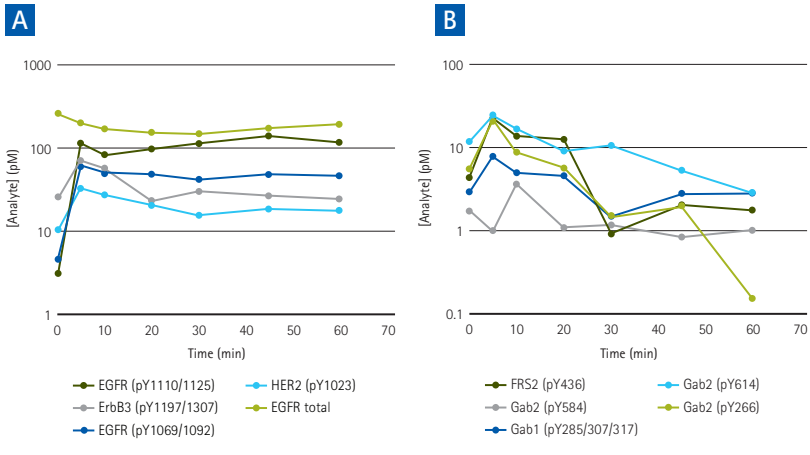


EGFR (red) expression in A431 cells is demonstrated using a Cy-3 conjugated primary antibody (Cat. No. 05-1047) via confocal immunocytochemistry, with nuclear staining (blue) and actin staining (green).

MILLIPLEX® MAP EpiQuant™ EGFR Signaling Pathway Panel

(Catalogue No. MPEQMAG-110K)

The ability to quantitatively analyze phosphorylation status of EGFR (ErbB) family members, as well as receptor-related intracellular proteins, is necessary for a thorough understanding of this pathway. This 22-plex immunoassay is ideal for absolute quantitation of ErbB pathway constituents, including phosphorylation sites on EGFR, ErbB2, ErbB3, and 16 other receptor related proteins. It also enables quantitation of total EGFR and a loading control (TAFII68) simultaneously in the same assay well. The Sample Preparation Kit (Cat. No. MPEQ-SP) is required before running any EpiQuant™ Panel.

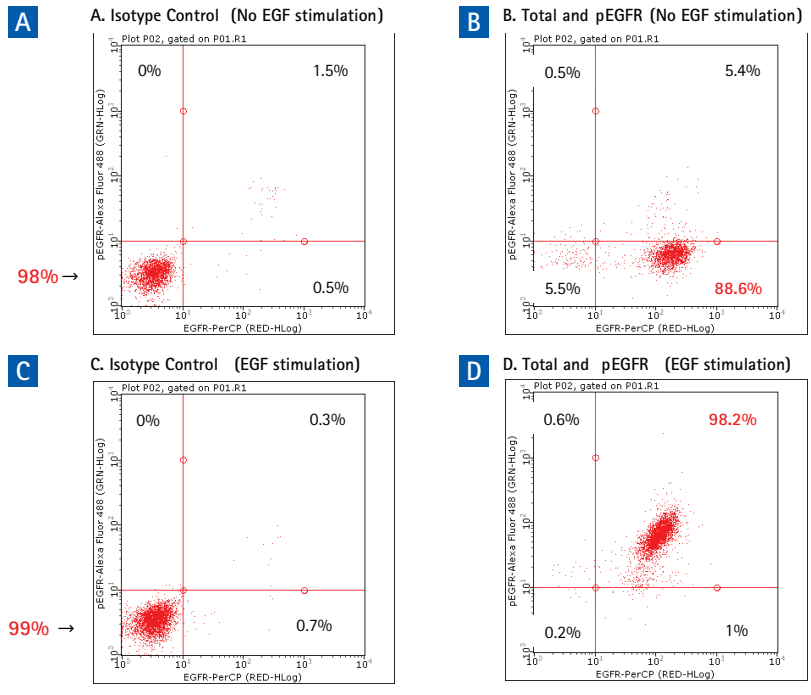


Time course of EGFR pathway protein phosphorylation shows persistent phosphorylation of EGF receptors (A) but more transient phosphorylation of other pathway proteins (B). All analytes, as well as total EGFR, were simultaneously detected in A431 cells treated with 100 ng/mL EGF. Values are internally normalized utilizing the TAFII68 loading control.

FlowCellect® EGFR RTK Activation Dual Detection Kit

(Catalogue No. FCCS025107)

Harness the power of multiparameter flow cytometry to detect the extent of EGF pathway activation by measuring the EGFR phosphorylation in relation to the total EGFR expression in any given cell population. The levels of both the total and phosphorylated protein can be measured simultaneously in the same cell, resulting in a normalized and accurate measurement of EGFR activation after stimulation, and offering more reliable detection of the phospho:total ratio within a mixed population of cells.



Dual parameter analysis of total and phospho-EGFR on A431 cells. Untreated cells stained with an isotype control (A) and both pEGFR-Alexa Fluor® 488 and Anti-EGFR-PerCP (B) expressed mainly unphosphorylated EGFR (88.6% of cells). However, once A431cells were stimulated with 100 ng/mL EGF, the percentage of cells with phosphorylated EGFR increased from 5% to 98% (compare double positive cells in (B) and (D)). Target specificity of phosphorylation was confirmed by simultaneous measurement of both total and phospho EGFR. A431 stimulated cells showed no activity when stained with an isotype control (C).

Key Products

Description	Catalogue No.
Antibodies	
Anti-EGF	07-1432
Anti-EGFR (cytoplasmic domain)	05-1047
Anti-EGFR, polyclonal	06-847
Anti-FGFR-4	07-2112
Anti-phospho-IGF-1R (Tyr1161/Tyr1165/Tyr1166)	ABE332
Anti-TGF-β Receptor, type I	06-1086
Anti-VEGF	ABS82
Kits and Assays	
FlowCellect® EGFR RTK Activation Dual Detection Kit	FCCS025107
MILLIPLEX® MAP EpiQuant™ EGFR Signaling Pathway Panel	MPEQMAG-110K
MILLIPLEX® MAP Total EGF Receptor MAPmate™ Assay	46-606
MILLIPLEX® MAP Human Angiogenesis/Growth Factor Panel	HAG1MAG-12K
Proteins	
PDGFR, active	14-467
Transforming Growth Factor-α, recombinant human	GF022

Cytokines and Chemokines

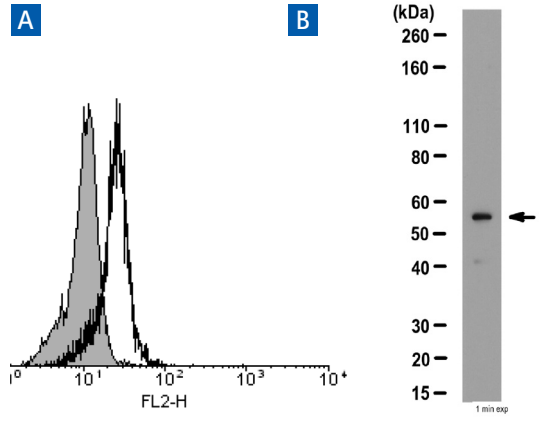
Cytokines and chemokines encompass large and diverse families of immunomodulating polypeptide regulators that are involved in mediating and regulating immunity, inflammation, and hematopoiesis. Secreted by specific cells in response to immune stimulus, these proteins mediate local intercellular communication and differ from hormones with respect to circulating concentrations and distribution. Unlike growth factor receptors, cytokine receptors generally lack identifiable catalytic activity. Cytokine receptors contain multiple cysteine residues and a conserved amino acid motif WSXWS (Trp-Ser-X-Trp-Ser) that functions in the recognition and binding of the ligand. Understanding cytokine and chemokine signaling can shed light on disease states, such as allergic reactions, inflammatory bowel disease (IBD), sepsis, and cancer.

Featured Products

Anti-Interleukin 17 Receptor β (IL-17Rβ)

(Catalogue No. 06-1071)

Interleukin-17 receptor β (IL-17Rβ) is a cytokine receptor that specifically binds to IL-17B and IL-17E, but does not bind to IL-17 or IL-17C. The IL-17 family of proinflammatory cytokines are produced by activated T cells, and high levels of IL-17 cytokines are associated with several chronic inflammatory diseases, including rheumatoid arthritis, psoriasis and multiple sclerosis. Overexpression of IL-17Rβ is linked to nonrecurrence after tamoxifen chemoprophylaxis in hormone receptor-positive breast cancer.

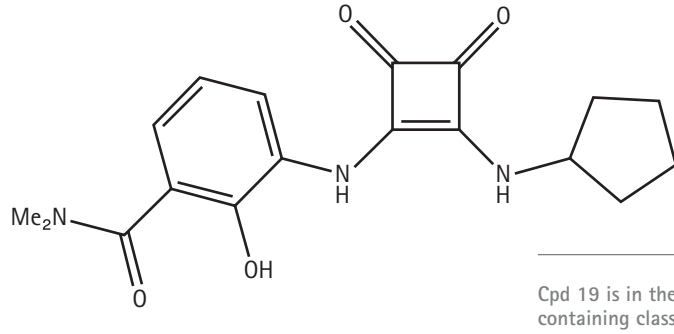


Flow cytometry analysis of human PBMCs with 1 µg Anti-IL-17 Receptor β (Cat. No. 06-1071, A). Western blot analysis of mouse testis lysate probed with Anti-IL-17 Receptor β (1 µg/mL, B). IL-17 Receptor β was detected at ~56 kDa.

CXCR2 Antagonist, Cpd 19

(Catalogue No. 239819)

This potent antagonist of chemokine receptor CXCR2 (IL8Rβ, IC_{50} = 8 nM) is a cell-permeable, cyclobutenedione derivative, ideal for chemical genetics studies of chemokine-activated chemotaxis of neutrophils toward sites of inflammation. Overexpression of CXCR2 and its ligand, IL-8, are associated with diseases caused by dysregulated inflammation, such as arthritis, asthma, and chronic obstructive pulmonary disease.



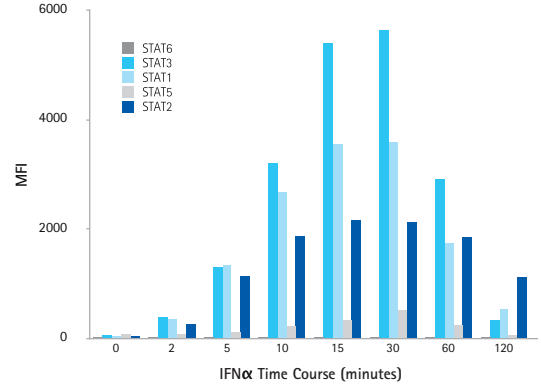
Cpd 19 is in the phenol-containing class of CXCR2 antagonists, which is the compound class that has been the most fruitful source of clinical candidates. Reported to inhibit CXCR2-mediated chemotaxis in a CXCR2 expressing cell line (IC_{50} = 145 nM), it exhibits good stability in human and rat liver microsomal preparations (>50% remaining after 30 min at 37°C).

MILLIPLEX® MAP STAT 5-Plex Panel

(Catalogue No. 48-610)

Multiple cytokine, hormone and growth factor receptors utilize JAK/STAT pathways for signaling. Quantifying the relative expression of STAT transcription factors is important for understanding the relationship between extracellular and nuclear signaling in normal physiological as well as pathological states including oncogenesis, immune responses and stem cell differentiation. This panel enables the simultaneous detection of multiple STAT proteins in a single well.

MILLIPLEX® MAP STAT Panel: IFNα Time Course

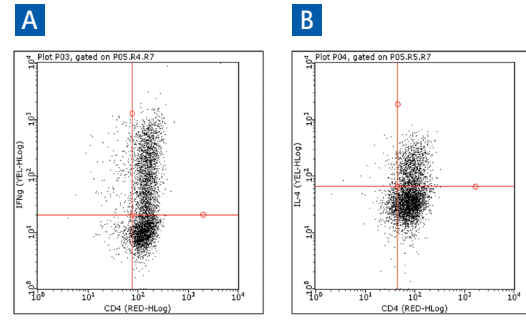


Increased levels of phosphorylated STAT1, 2, 3, 5 and 6 in stimulated cells compared to their unstimulated counterparts as detected using the MILLIPLEX® MAP Human STAT 5-Plex Panel. Phosphorylated STAT proteins were simultaneously detected in HeLa cells treated with 2,000 U/mL IFNα for 0, 2, 5, 10, 15, 30, 60, and 120 minutes.

FlowCellect® Mouse TH1/TH2 Identification Kit

(Catalogue No. FCIM025137)

FlowCellect® kits for intracellular cytokine assays are completely optimized and validated for flow cytometers with blue and red lasers. The kits include all necessary reagents and buffers, including a fixable viability dye. The FlowCellect® Mouse TH1/TH2 Identification Kit provides an easy way to evaluate the profile of an immune response, by detecting IFN-γ and IL-4 expression in mouse TH1 and TH2 CD4+ T-cells.



Cytokine expression in Th1 and Th2 cells detected using flow cytometry. Th1- and Th2-differentiated Cd4+ mouse T cells were stained with the FlowCellect® Mouse TH1/TH2 Identification Kit. Double-positive cells (in the upper right quadrant of each plot) represent live, CD4+ lymphocytes that also express IFNγ (Th1-differentiated cells, A) or IL-4 (Th2-differentiated cells, B).

Key Products

Description	Catalogue No.
Antibodies	
Anti-IL-16, clone 14.1	MABF29
Anti-Interleukin 10 Receptor α (IL-10Rα)	06-1067
Anti-Interleukin 17 Receptor β (IL-17Rβ)	06-1071
Anti-Interleukin 21 (IL-21)	06-1074
Anti-Interleukin 23 (IL-23) p40, clone 2G6	04-1582
Anti-Interleukin 23 α (IL-23α)	06-1079
Anti-Interleukin 26 (IL-26)	06-1081
Anti-Interleukin 27 (IL-27)	06-1082
Anti-Interleukin 4 (IL-4)	06-1083
Anti-TNFα	04-1114
Anti-TNF Receptor	MAB3216
Small Molecule Inhibitors	
CXCR2 Antagonist, Cpd 19	239819
CXCR4 Antagonist I, AMD3100	239820
CXCR4 Antagonist II	239821
Kits and Assays	
FlowCellect® Mouse TH1/TH2 Identification Kit	FCIM025137
MILLIPLEX® MAP Human Soluble Cytokine Receptor Panel	HSCRMAG-32K
MILLIPLEX® MAP Human STAT 5-Plex Panel	48-610MAG
MILLIPLEX® MAP TGFβ Signaling Pathway 6-Plex Panel	48-614MAG
Proteins	
TNF-α, recombinant rat	GF046
Interleukin-11, recombinant	IL011

G-Protein Coupled Receptors (GPCRs)

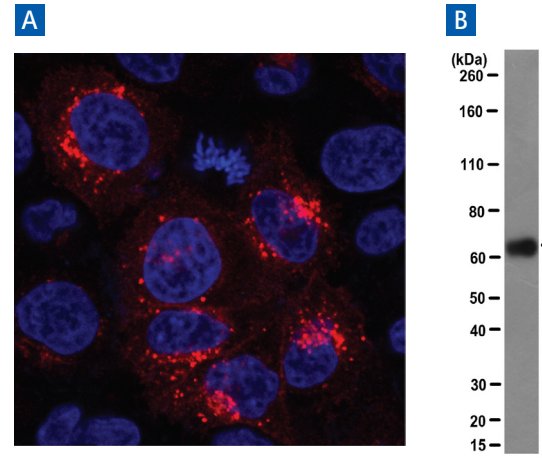
GPCRs are one family of specialized transmembrane receptors proteins that facilitate communication with the extracellular environment. A receptor's main function is to recognize and respond to molecules on the extracellular domain and initiate a response or signaling cascade via G protein signaling from the intracellular domain. GPCR-associated signaling pathways control numerous essential functions in all tissues and are ubiquitous throughout the animal kingdom. Merck Millipore provides a wide range of products for identification and quantification of surface expression levels and activity levels of GPCRs.

Featured Products

Anti-GPR177, clone YJ5

(Catalogue No. MABS87)

Wnt family proteins are secreted signaling proteins that regulate differentiation and development, and frequently regulate gene expression in tissues at sites distant from the point of secretion. GPR177 (WLS, EVI), the mouse ortholog of *Drosophila* Wntless, is a G protein-coupled receptor that binds to Wnt and helps mediate its long-range effects. GPR177 is essential for the patterning of the anterior-posterior axis during mammalian development and is activated by β-catenin and LEF/TCF-dependent transcription. Upon GPR177 activation, Wnt binds to and modifies the subcellular distribution of GPR177, leading to a feedback regulatory mechanism involving Wnt expression and signaling.

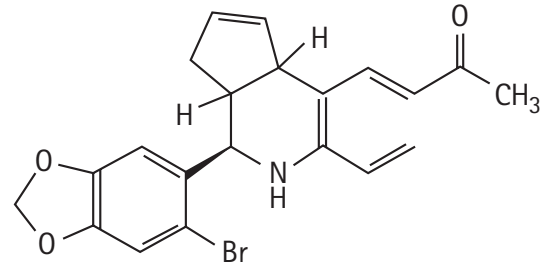


GPR177 detected on subcellular membranes in HeLa cells transfected with human GPR177 using immunocytochemistry with Anti-GPR177, clone YJ5 (1:50, Cat. No. MABS87, A). GRP177 was visualized using a Goat Anti-Mouse IgG secondary antibody conjugated to Alexa Fluor® 594 dye (Red). Nuclei are stained with DAPI (Blue). (Data courtesy of Prof. D.M. Virshup, Program in Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School.) Western blot analysis of HeLa cell lysate probed with Anti-GPR177, clone YJ5 (1:5,000, B). GPR177 was detected at ~62 kDa.

GPR30 Agonist, G-1

(Catalogue No. 371705)

Although estrogen is best known for binding to nuclear receptors to regulate gene expression, it also binds to GPR30, a GPCR localized to the endoplasmic reticulum, to mediate rapid cellular responses to injury via the PKA pathway. Use this high-affinity agonist for GPR30 to study the clinically important nongenomic roles of estrogen. G-1 specifically competes with estrogen binding to GPR30 (Ki = 11 nM), but does not bind the classical nuclear-residing estrogen receptors, ERα and Erβ.

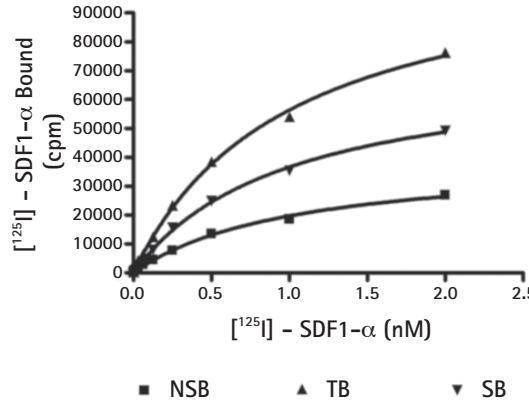


A cell-permeable, nonsteroidal, dihydroquinoline compound, G-1 has been shown to stimulate GPR30-mediated cellular PI3K activation and calcium mobilization (EC_{50} = 2 nM in COS7 cells) and inhibit migration of SKBr3 and MCF-7 cells toward chemoattractants (IC_{50} = 0.7 and 1.6 nM, respectively).

ChemiSCREEN™ CXCR4 Membrane Preparation

(Catalogue No. HTS004M)

C-X-C chemokine receptor type 4 (CXCR4) transduces a signal by increasing intracellular calcium and is vital for cell trafficking during development. Stromal-derived factor 1α (SDF-1α) binds to CXCR4 expressed on hematopoietic and lymphopoietic cells, and directs their trafficking to sites of inflammation. CXCR4 is expressed on several tumor cell lineages and may direct metastasis to sites of SDF-1α expression. ChemiSCREEN™ CXCR4 membrane preparations are made from stable cell lines that express high levels of recombinant CXCR4 and are ideal tools for screening for CXCR4 and SDF-1α antagonists.



Competitive radioligand binding assay results using the ChemiSCREEN™ CXCR4 membrane preparation and 125I-labeled SDF-1α and unlabeled SDF-1α demonstrate total binding (TB), nonspecific binding (NSB) and specific binding (SB).

Key Products

Description	Catalogue No.
Antibodies	
Anti-GPR177, clone YJ5	MABS87
Anti-GRK 2/3 (βARK 1/2)	05-465
Small Molecule Inhibitors	
GPR30 Agonist, G-1	371705
S1P1 Receptor Agonist, SEW2871	567733
SB 290157	559410
Kits and Assays	
ChemiSCREEN™ CXCR4 Membrane Preparation	HTS004M
FlowCellect® Chemokine Receptor CXCR4 Surface Kit	FCXR400423

Ion Channels

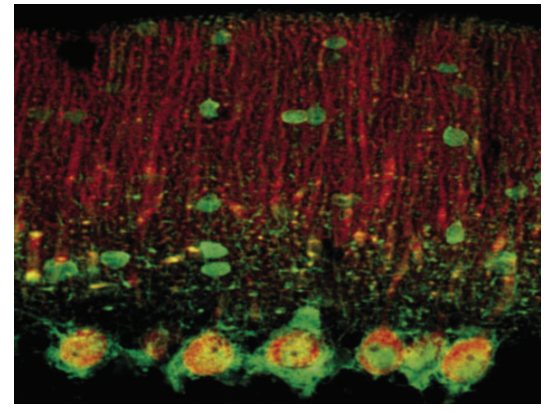
Ion channels, the ion-permeable pores in lipid bilayers, constitute a large family of pore-forming transmembrane proteins. They open and close in response to specific stimuli and facilitate the transport of charged molecules and control the voltage across the plasma membrane by facilitating the flow of ions down an electrochemical gradient. Ion channels enable rapid signaling in electrically excitable cells, such as neurons. In the open conformation, ions are tightly bound by the channel, which discriminates between sizes and charges of the pervading molecules. The conformational change between closed and open state (gating) is controlled by external modulators such as ligand binding, voltage, second messengers, and G-proteins.

Featured Products

Anti-Calcium Channel, Voltage-Gated α 1C (Cav1.2)

(Catalogue No. AB5156)

Voltage-sensitive calcium channels mediate the influx of calcium ions into the cell upon membrane depolarization and are involved in processes such as muscle contraction, neurotransmitter release, cell motility, and cell death. The isoform α-1c gives rise to L-type (long-lasting) calcium currents. L-type channels are sensitive to dihydropyridines, which are frequently used to treat hypertension. Expression is seen in brain, heart, jejunum, ovary, pancreatic β-cells, and vascular smooth muscle.



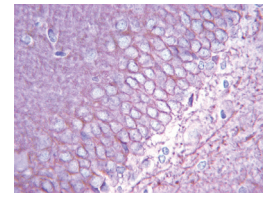
Immunocytochemical analysis of the rat cerebellum using Anti-Calcium Channel, Voltage Gated α 1C. This antibody positively stains Purkinje cells (red). Photo courtesy of Dr. W. Hartig and J. Grosche, Leipzig University, IZKF.

Anti-K^+/Cl^- Cotransporter (KCC2), clone N1/12

(Catalogue No. MABN88)

KCC2, a cation-chloride cotransporter, is proposed to act as the main chloride extruder to promote fast hyperpolarizing postsynaptic inhibition in the brain. KCC2 is expressed at high levels in neurons throughout the nervous system and immunofluorescence shows that the protein is localized at inhibitory synapses of the spinal cord. Studies in mice have shown that KCC2 reduces GABA's inhibitory signaling, resulting in motor defects, epilepsy, and anxiety-like behavior.

A



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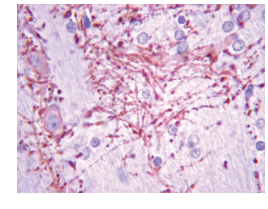
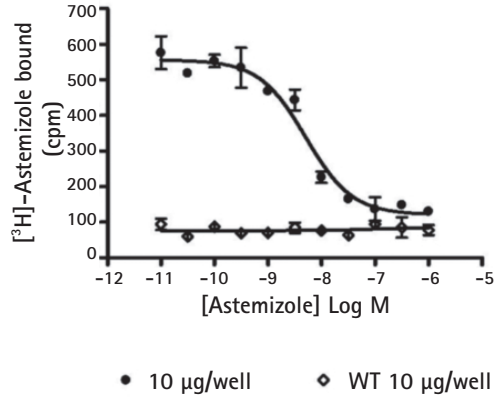


KCC2 is expressed in the neuronal membranes and synapses of the rat brain. Immunohistochemistry analysis of paraffin-embedded rat hypothalamus (A) and cortex area A-3 (B) using Anti-K+/Cl- Cotransporter (KCC2), clone N1/12 (1:300, Cat. No. MABN88). Reactivity was detected using the IHC Select® Detection Kit (Cat. No. DAB050).

PrecisION® Membrane Preparation Recombinant hERG Potassium Ion Channel

(Catalogue No. CYL4039)

The human ether-a-go-go-related gene (hERG) encodes a voltage-gated potassium channel which mediates cardiac action potential repolarization in the mammalian heart. Many drugs and drug candidates can block the hERG channel, causing cardiotoxicity, making hERG screening a critical preclinical study. This membrane preparation is made from the PrecisION® recombinant hERG ion channel stable cell line; like all PrecisION® cell lines, it is biophysically and pharmacologically validated using electrophysiology.



Competition binding for hERG membrane preparation versus wild-type HEK293 membrane preparations using [3H]-astemizole, a known hERG antagonist.

Key Products

Description	Catalogue No.
Antibodies	
Anti-Acid Sensitive Ion Channel 1	AB5674P
Anti-Cav1.2 Calcium Channel, Voltage Gated α 1C	AB5156
Anti-Cav1.2 Calcium Channel, clone L57/46	MAB13170
Anti-K+/Cl- Cotransporter (KCC2), clone N1/12	MABN88
Anti-Potassium Channel Kv1.2, clone K14/16	MABN77
Anti-Sodium channel Nav1.7, clone N68/6	MABN41
Kits and Assays	
PrecisION® Membrane Preparation Recombinant hERG Potassium Ion Channel	CYL4039

Cytosolic Signaling

Cytosolic signaling proteins play crucial roles in cell cycling, targeted proteolysis, protein trafficking, cytoskeletal organization, and gene expression. Mutations in or misregulation of these proteins are associated with initiation, promotion, and progression of various diseases including cancer, diabetes and rheumatoid arthritis. Cytosolic signaling is dominated by protein phosphorylation and dephosphorylation by protein kinases and protein phosphatases, which represent the most common targets of therapeutics, accounting for over 25% of drug discovery programs worldwide. A leader in cell signaling, Merck Millipore is building the most comprehensive selection of tools for studies of phosphorylation and other post-translational modifications.

Post-Translational Modifications

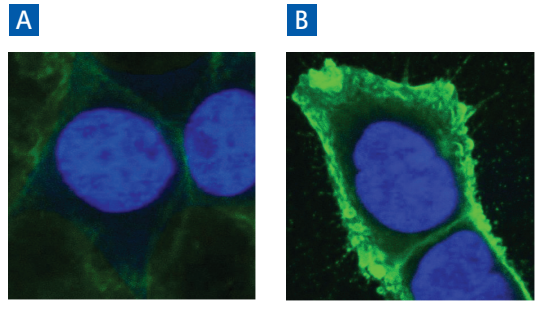
Many proteins, like insulin, are synthesized as inactive precursors and must be proteolytically cleaved for activation. However, there are several other modifications such as acetylation, methylation, phosphorylation, and ubiquitination that impact protein function and activity. As a result, the analysis of proteins and their post-translational modifications is particularly important for the study of normal and disease-associated processes.

Featured Products

4G10® Platinum, Anti-Phosphotyrosine

(Catalogue No. 05-1050)

Well known for its sensitive recognition of multiple phosphotyrosines on numerous substrates, 4G10® has been validated by thousands of researchers in virtually every application and tyrosine target. To improve 4G10® further, we pooled it with the next most highly regarded phosphotyrosine antibody, clone PY20, to make 4G10® Platinum, enabling more sensitive detection of more substrates than 4G10® alone.

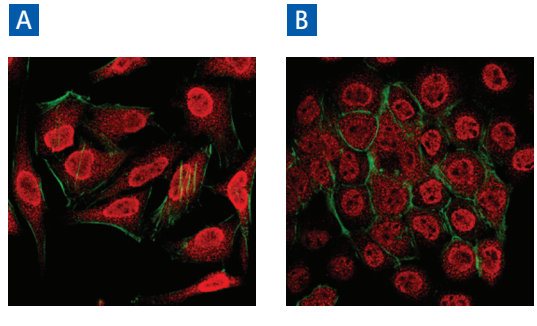


Confocal immunofluorescence using 4G10® Platinum Anti-phosphotyrosine of EGF-treated A431 cells (right) versus untreated (left) demonstrate activation via phosphorylation (green). Nuclear staining was achieved using DAPI (blue).

Anti-Ubiquitin K11 Linkage, clone 2A3/2E6

(Catalogue No. MABS107)

Ubiquitin is a small protein that can be covalently attached to proteins either as a monomer or as a polymer. Lysine11 (K11)-linked ubiquitin chains regulate mitotic protein degradation by acting as a specific substrate for the anaphase-promoting complex (APC), which has E2 and E3 ubiquitin ligase activity. Antibodies specific to the K11-linkage have shown increased formation of K11 chains in mitotic human cells in the presence of APC substrate degradation.

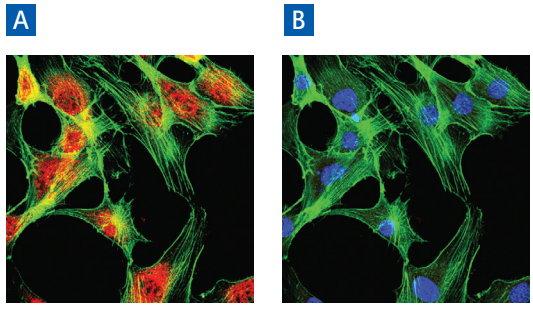


Immunocytochemistry analysis of HeLa (A) and A431 (B) cells using 1:500 Anti-Ubiquitin K11 linkage, clone 2A3/2E6 (Cat. No. MABS107, Red). Actin filaments have been labeled with Alexa Fluor® 488 dye-Phalloidin (green). Anti-Ubiquitin K11 stains both the cytosol and nucleus (with intense staining on nucleus).

Anti-Ubiquitin, Lys48-Specific, clone Apu2

(Catalogue No. 05-1307)

Polyubiquitin chains linked through the Lys48 residue of ubiquitin are most commonly associated with proteins targeted for proteosomal degradation. Misregulation of protein metabolism can lead to unchecked proliferation and tumorigenesis. Visualize protein turnover with this robust anti-ubiquitin (Lys48-specific) monoclonal antibody.

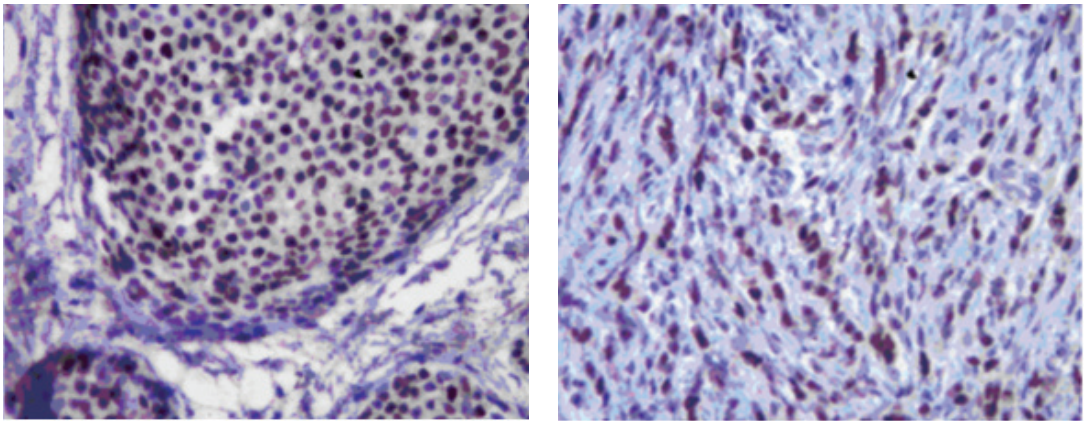


Confocal IF using Cat. No. 05-1307 shows Lys48-linked ubiquitin, marking proteins for degradation. Ubiquitin signal (red, left panel) is strongest in the nucleus (blue, right panel).

Anti-phospho-Estrogen Receptor (ER) α (Ser305), clone 124.9.4

(Catalogue No. 05-922R)

Resistance to the ER-binding drug, tamoxifen, is one of the major challenges in breast cancer treatment. Phosphorylation of ERα at serine 305 (ERαS305-P) by protein kinase A (PKA) leads to an activation of ERα and to transcription of ERα-responsive genes after tamoxifen treatment, leading to tamoxifen resistance[1,2]. Clinical studies also show that ERαS305-P may be a good biomarker to identify patients unlikely to respond to tamoxifen[3,4]. Our extensively validated antibody for ERαS305-P specifically reacts with breast cancer tissue from samples from ER-positive patients as shown by immunohistochemistry.



Immunohistochemistry analysis of ductal carcinoma using phospho-ERα (Ser305), clone 124.9.4 (1:200). Nuclear reactivity (as seen here in both images) was only observed when the cancer case was fully involved with ER only and with a high proliferation rate combined with a high S-phase and DNA index.

1. Zwart W et al. PKA-induced resistance to tamoxifen is associated with an altered orientation of ERalpha towards co-activator SRC-1. EMBO J. 2007 Aug 8;26(15):3534-44.

2. Wang RA et al. P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. EMBO J. 2002 Oct 15;21(20):5437-47.

3. Holm C et al. Phosphorylation of the oestrogen receptor alpha at serine 305 and prediction of tamoxifen resistance in breast cancer. J Pathol. 2009 Feb;217(3):372-9.

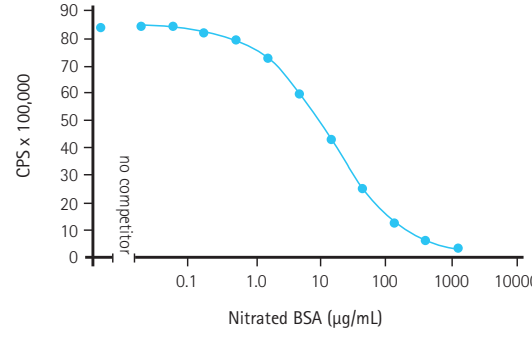
4. Kok M et al. PKA-induced phosphorylation of ERα at serine 305 and high PAK1 levels is associated with sensitivity to tamoxifen in ER-positive breast cancer. Breast Cancer Res Treat. 2011 Jan;125(1):1-12.

Featured Products

Nitrotyrosine ELISA

(Catalogue No. 17-376)

Reactive oxygen species (ROS), such as nitric oxide (NO•), superoxide (O2•), peroxynitrite ($ONOO^-$), and hydroxyl radical (OH^-) can cause nitration of tyrosine residues. The nitrotyrosine assay kit with chemiluminescence detection is a competitive ELISA for quantifying tyrosine nitration. The kit includes all required reagents, including white, high-binding 96-well plates, nitrated BSA standard, anti-nitrotyrosine antibody, LumiGLO® detection substrate, and wash buffers. The assay has a wide dynamic range and high precision, making it a valuable tool for studying oxidative stress.

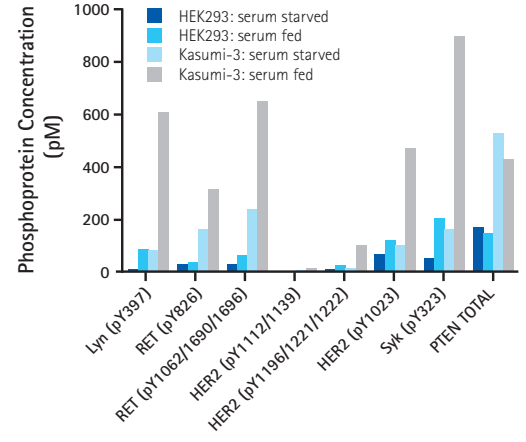


The linear range of the nitrotyrosine ELISA encompasses two orders of magnitude.

MILLIPLEX® MAP EpiQuant™ Human Receptor Signaling Pathway Panel

(Catalogue No. MPEQMAG-104K)

Receptor phosphorylation is one of the key steps in initiating signal transduction from the membrane. Use this 27-plex immunoassay based on EpiQuant™ technology for absolute quantitation of 11 receptor signaling-related protein targets as well as the degree of phosphorylation at specific sites on these proteins. These total and phosphorylated proteins include HER2, HER3, VEGFR1 and VEGFR2, among others. We have validated this panel for the analysis of various stimulated tissue culture lysates.



Serum starvation decreases receptor phosphorylation (but not PTEN levels) in HEK293 and Kasumi-3 cellular lysates. Phosphorylated proteins were measured in parallel in lysates from serum-starved and serum-fed cells. Total protein levels were simultaneously assessed in the same well for each of the listed phosphorylation targets and used to normalize expression values.

Key Products

Description	Catalogue No.
Antibodies	
Anti-AMPylated Threonine	09-890
Anti-dimethyl-Arginine, asymmetric (ASYM25)	09-814
Anti-modified Citrulline	07-2168
Anti-O-GlcNAc, clone 1F5.D6(14)	05-1246
Anti-Sulfotyrosine, Clone Sulfo-1C-A2	05-1100
Focal Adhesion Pathway Explorer Antibody MiniPack	15-113
mTOR Phosphorylation Pathway Explorer Antibody MiniPack	15-105
Kits and Assays	
MILLIPLEX® MAP EpiQuant™ Phosphothreonine Cell Signaling Panel 1	MPEQMAG-103K
MILLIPLEX® MAP Heat Shock Protein 5 Plex	48-615MAG
MILLIPLEX® MAP Multi-Pathway 9-Plex	48-680MAG

Kinases, Phosphatases and Their Substrates

Phosphate groups are key posttranslational modifications to proteins in signaling pathways. Protein phosphorylation and dephosphorylation regulate normal physiological and pathological processes that contribute to complications, including cancer, central nervous system pathologies, diabetes, pain, inflammation, autoimmune diseases, and cardiotoxicity. Therefore, the responsible modifying enzymes (kinases and phosphatases) and their substrates have been the focus of therapeutic modulation, chemical genetics experiments and quantitative detection for the elucidation of signaling networks and dynamics.

Featured Products

Insulin Receptor Substrate 1 (IRS1) Antibodies

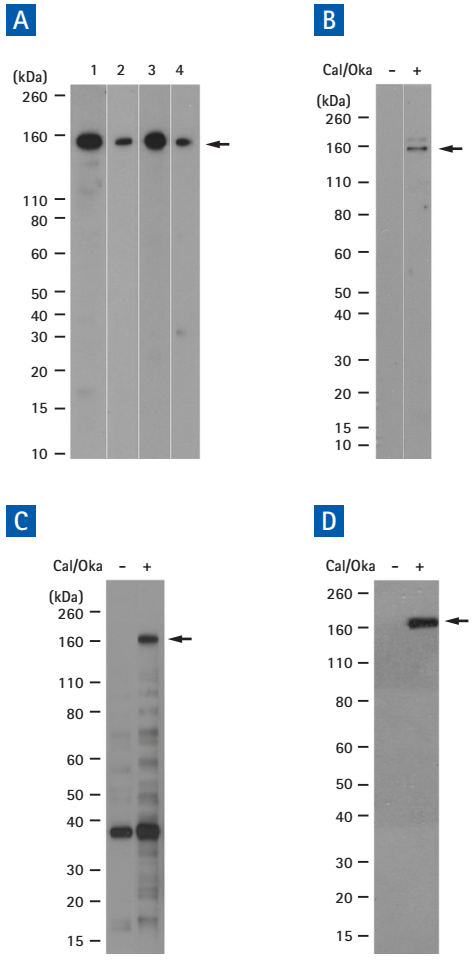
Unphosphorylated IRS1 detection:

Anti-IRS1, clone 58-10C-31 (Catalogue No. AB5156)

Phosphorylated IRS1 detection:

Anti-phospho-IRS1 (Ser632), clone 5.3.3 (Catalogue No. 05-1568)
Anti-phospho IRS1 (Ser522), clone 17.5.2 (Catalogue No. 05-1921)
Anti-phospho IRS1 (Ser318), clone 3.1.1 (Catalogue No. MABS138)

IRS1 transmits insulin signals via metabolic and mitogenic pathways. IRS1 is heavily phosphorylated on both serine and tyrosine residues. These phosphorylated tyrosines enable IRS to act as a docking protein that binds SH2 domains of such proteins as PI3 Kinase (phosphatidylinositol 3-kinase) and GRB2, resulting in activation. Over expression and phosphorylation of serine is associated with insulin resistance and breast cancer. Ser302 is phosphorylated following insulin stimulation. Ser307, phosphorylated by JNK and IKK, is a key regulatory site that appears to disrupt the IRS1/IR interaction and inhibits insulin-mediated activation of the PI3 kinase and MAPK pathways, and Ser636/639 is known to be phosphorylated by p70S6K downstream of mTOR in a negative feedback loop.



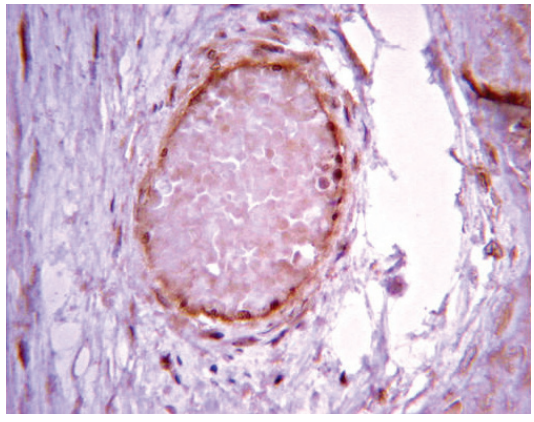
A. Western blot analysis of 3T3/A31 (lane 1),3T3/L1 (lane 2), L6 (lane 3), and MCF7 (lane 4) lysates probed with Anti-IRS1, clone 58-10C-31 (1:1000) showed IRS1 as a band at ~160 kDa.

B - D. IR/IRS1 transfected CHO cells with (+) and without (-) phosphatase inhibitors calyculin A and okadaic acid. Lysates were subjected to Western blotting and probed with: (B) Anti-phospho-IRS1 (Ser632) (Cat. No. 05-1568), (C) Anti-phospho IRS1 (Ser522), clone 17.5.2 (Cat. No. 05-1921), or (D) Anti-phospho IRS1 (Ser318), clone 3.1.1 (Cat. No. MABS138). Phospho-IRS1 was detected at ~160 kDa.

Anti-Calcineurin

(Catalogue No. 07-1490)

Calcineurin/PP2B is a serine/threonine protein phosphatase involved in a variety of calcium-mediated cellular responses. Calcineurin/PP2B has been linked to Pmk1 MAP kinase and phosphoinositide (PI) pathways, as well as to members of the small GTPase Rab/Ypt family and Type II myosin. Furthermore, calcineurin helps transduce extracellular signals to the nucleus by dephosphorylating members of the NFAT family of transcription factors.



Calcineurin immunohistochemistry reveals morphology of paraffin-embedded human vascular tissue. Tissue was pretreated with citrate pH 6 for antigen retrieval.

InhibitorSelect™ 96-Well Tyrosine Kinase and Phosphatase Inhibitor Library IV

(Catalogue No. 539747)

This library consists of 83 pharmacologically active, well-documented, cell-permeable, potent and reversible protein kinase and phosphatase inhibitors; the majority of kinase inhibitors are ATP-competitive. Use this library to advance target identification in drug discovery, biochemical pathway analysis, screening new protein kinases/phosphatases, high content screening and more.

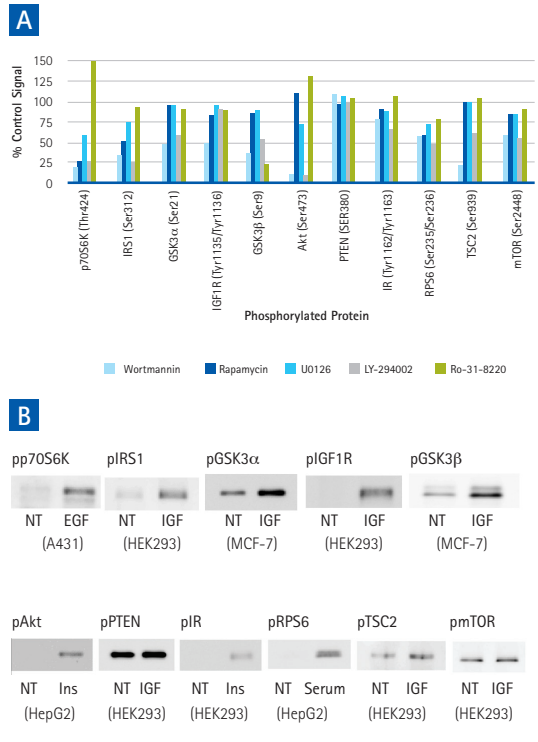
Enzyme Family	Number of Inhibitors in Library
Receptor Tyrosine Kinase (RTK)	37
Tyrosine Kinase (TK)	27
Tyrosine Kinase-like (TKL)	6
PKA, PKG, PKC families (AGC)	1
CDK, MAPK, GSK-3, CLK families (CMGC)	3
Receptor Tyrosine Phosphatase (RTP)	1
Tyrosine Phosphatase (TP)	8

Kinases and phosphatases from many enzyme families are inhibited by structurally diverse small molecules in InhibitorSelect™ 96-Well Tyrosine Kinase and Phosphatase Inhibitor Library IV.

MILLIPLEX® MAP Phosphorylated Akt/mTOR 11-Plex Panel

(Catalogue No. 48-611MAG)

As nearly all of the players in the Akt/mTOR signaling pathway are coordinately regulated by phosphorylation, understanding the role of this pathway in normal physiological processes and in diseases such as cancer and diabetes requires the ability to simultaneously measure phosphorylation status of multiple protein targets. The MILLIPLEX® MAP Akt/mTOR 11-plex Panel has been successfully used to simultaneously quantify multiple pathway proteins in cancer cell lines (HepG2, HEK293, and MCF7) as well as in human breast cancer tissue samples.



(A) Effect of inhibitors on phosphorylation of Akt/mTOR pathway proteins in HepG2 cells. Cells were pre-treated with 0.1 µM wortmannin, 0.1 µM rapamycin, 10 µM U0126 (MEK1/2 inhibitor), 50 µM LY-294002 (PI3K inhibitor), or Ro-31-8220 (PKC and GSK3α/β inhibitor) for 30 minutes prior to the addition of 10 µg/mL insulin for 15 minutes.

(B) Validation by immunoprecipitation (IP) /Western blotting. IP of phosphoproteins in cell lines treated with either insulin or IGF1 was performed with capture beads and proteins detected by Western blotting with the biotinylated detection antibodies.

Key Products

Description	Catalogue No.
Antibodies	
Akt/mTOR/S6K Pathway Explorer Antibody MiniPack	15-104
Anti-Calcineurin	07-1490
Anti-IRS1, Alexa Fluor® 488 conjugated	16-257
Anti-IRS1, clone 58-10C-31	05-784R
Anti-IRS-2, clone 9.5.2	MABS15
Anti-MAPK 1/2	ABS44
Anti-mouse Tks4	09-260
Anti-phospho IRS1 (Ser318), clone 3.1.1	MABS138
Anti-phospho IRS1 (Ser522), clone 17.5.2	05-1921
Anti-phospho-IRS1 (Ser632), clone 5.3.3	05-1568
Anti-phospho-p38 (Thr180/Tyr182), clone 6E5.2	MABS64
Small Molecule Inhibitors	
Akt Inhibitor XV, Isozyme Selective	124034
Aurora Kinase Inhibitor VI	189410
InhibitorSelect™ 96-Well Tyrosine Kinase and Phosphatase Inhibitor Library IV	539747
JNK Inhibitor III, SR-3306	420147
SB 203580	559389
U0126	662005
Y-27632	688000
Kits and Assays	
FlowCellect® Multi-STAT Activation Profiling Kit	FCCS025550
FlowCellect® PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection kit	FCCS025100
FlowCellect® PI3 Kinase-mTOR Signaling Cascade Kit	FCCS025210
MILLIPLEX® MAP 10-Plex MAPK/SAPK Signaling Kit – Phosphoprotein	48-660MAG
MILLIPLEX® MAP Phosphorylated Akt/mTOR 11-Plex Panel	48-611MAG
Proteins	
AMPK (α1, β1, γ1), active	14-840

G-Proteins and Second Messengers

Ligand binding of G protein-coupled receptors (GPCRs) induces a conformational change allowing the receptor to function as a switch, alternating between an inactive guanosine diphosphate (GDP)- and active guanosine triphosphate (GTP)-bound states. These different states regulate signal transducing molecules, such as secondary messengers and kinases, that ultimately impact downstream cellular processes. The disruption of G-protein signals have been implicated in several diseases such as diabetes, cardiovascular defects and certain forms of cancer.

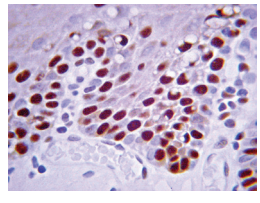
Second messengers are molecules that relay signals received and processed by cell surface receptors. They amplify these signals to cause a biological effect. Commonly recognized second messengers include cAMP, cGMP, IP3, DAG, and nitric oxide.

Featured Products

Anti-Ras, pan

(Catalogue No. 05-1071)

The product of RAS, the first human oncogene identified, is a small G-protein that activates the Erk/MAPK kinase pathway by activating Raf, and also activates PI3 Kinase (PI3K) and RalGDS. In its oncogenic, mutated state, Ras is unable to hydrolyze GTP to GDP, thus staying in an active state. Mutations in Ras have been found in a large percentage of all human cancers.

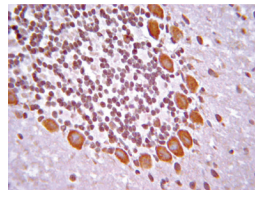


Immunohistochemistry staining of stratified human squamous epithelium using Anti-Ras (Cat. No. 05-1071) and the IHC Select® Detection System.

Anti-cAMP

(Catalogue No. 07-1497)

Cyclic adenosine monophosphate (cAMP) is an intracellular product of ATP catalysis by adenylate cyclase in a downstream response to GPCR activation by extracellular ligands. cAMP in turn activates intracellular kinases, thus acting as a second messenger for signal transduction across the cell membrane. For example, cAMP regulates the effects of glucagon, adrenaline and calcium flux through ion channels. Since it is affected directly by GPCR activation, cAMP is can be used to monitor GPCRs in the discovery of GPCR-modulating drugs.

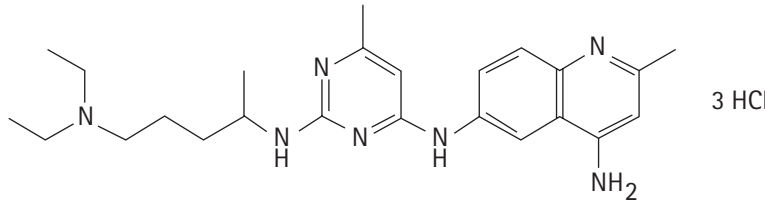


cAMP detected in paraffin-embedded rat cerebellum tissue using immunohistochemical staining with Anti-cAMP (1:100, Cat. No. 07-1597). Tissue was prepared using heat-induced epitope retrieval in citrate buffer, pH 6.0. Reactivity was detected using the IHC Select® Detection Kit (Cat. No. DAB050).

Rac1 Inhibitor

(Catalogue No. 553502)

Rac1 is a small G protein that signals to the cytoskeleton to modulate cell polarity, adhesion and motility in order to regulate cell cycle, cell migration and epithelial differentiation. This Rac1 inhibitor specifically and reversibly inhibits Rac1 GDP/GTP exchange activity by interfering with binding by the Rac-specific GEFs (guanine nucleotide exchange factors) Trio and Tiam1 (IC_{50} ~50 µM).

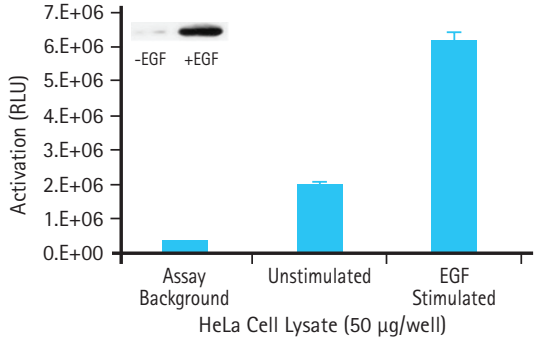


A cell-permeable pyrimidine compound, Rac1 inhibitor effectively blocks Rac1-mediated cellular functions in NIH3T3 and PC-3 cells (effective dose ~50 to 100 µM).

Ras Activation ELISA Kit

(Catalogue No. 17-497)

The Ras family of G proteins are GDP/GTP-regulated binary switches that can control cell growth, proliferation, differentiation, or survival. Because it is a key regulator in several tumor types, Ras has been a popular target for cancer research and anti-cancer therapeutics for the past two decades for both academic and pharmaceutical research. This kit exploits the specific binding of Raf to activated, GTP-bound Ras. Affinity purification using agarose-conjugated GST-Ras binding domain (GST-RBD) of Raf is followed by anti-Ras ELISA.

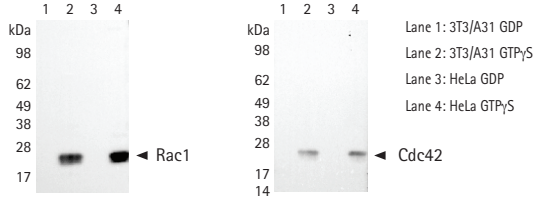


EGF-stimulated HeLa cell lysates show elevated levels of activated Ras compared with the basal levels in unstimulated samples.

Rac1/Cdc42 Activation Assay Kit

(Catalogue No. 17-441)

Rac1 and Cdc42 are small G-proteins that modulate cytoskeletal organization to affect cell migration, polarity, growth and other processes. This kit effectively detects Rac1 and Cdc42 activity in cell lysates. This assay uses the downstream effector of Rac1/Cdc42, p21-activated protein kinase (PAK1), to isolate the active, GTP-bound Rac1/Cdc42 from the sample. The p21 binding domain (PBD) of PAK1 is expressed as a GST-fusion protein and coupled to agarose beads. After precipitation, activated Rac1/Cdc42 can be detected by Western blotting.



Rac1 is activated by GTP but not GDP in 3T3/A31 and HeLa cells. Lysates were incubated with either GDP or GTPγS. Activated Rac1 or Cdc42 were isolated using the activation assay kit (Cat. No. 17-441) and detected by Western blotting with Anti-Rac1 (left) or Anti-Cdc42 (right).

Key Products

Description	Catalogue No.
Antibodies	
Anti-cAMP	07-1497
Anti-Ras	05-1071
Small Molecule Inhibitors	
Dynamin Inhibitor I, Dynasore	324410
InSolution™ Rac1 Inhibitor	553508
Rac1 Inhibitor	553502
Kits and Assays	
Rac1/Cdc42 Activation Assay Kit	17-441
Ras Activation ELISA Kit	17-497

Lipid Signaling

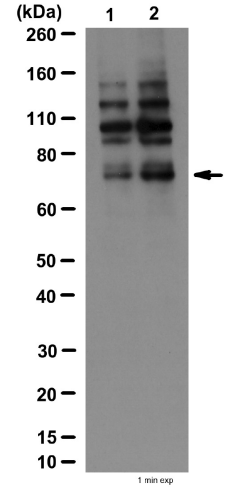
Bioactive lipids, generated during remodeling of membrane lipids by activated lipases, serve as intra- and extracellular mediators in cell signaling. Although the interaction between a lipid-based messenger and a cellular receptor can mediate downstream signaling cascades similar to other ligand-receptor relationships, lipid signaling is unique in that lipids can freely diffuse through cellular membranes. Additionally, lipid messengers can neither be stored in vesicles nor do many lipid signaling molecules circulate freely in solution. As a result, lipid messengers are often synthesized on demand at the site of action or are bound to lipid carrier proteins in serum.

Featured Products

Anti-phospho-Grb10 (Ser501/Ser503)

(Catalogue No. 07-1520)

Growth factor receptor-bound protein 10 (Grb10) was recently identified as substrate of mTOR and therefore a downstream target of lipid signaling via PI3 kinase. It mediates signals from a number of activated receptor tyrosine kinases, growth factor receptors, and intracellular molecules. Grb10 downregulates insulin signaling via feedback inhibition of the PI3 kinase pathway, potentially acting as a tumor suppressor.

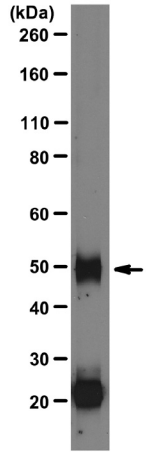


Phosphorylated Grb10 is more abundant in insulin-treated fibroblasts compared to untreated cells. Western blot analysis of untreated (lane 1) and insulin-treated (lane 2) mouse embryonic fibroblast cell lysates probed with Anti-phospho-Grb10 (Ser501/Ser503) (1 µg/mL). This antibody detected Grb10 at ~66 kDa.

Anti-Inositol Hexakisphosphate Kinase 2 (IP6K2)

(Catalogue No. 05-1545)

Generally, inositol phosphates are second messengers in the phosphatidylinositol lipid signaling pathway. IP6K2 converts one of these second messengers, inositol hexakisphosphate (InsP6), to diphosphoinositol pentakisphosphate (InsP7/PP-InsP5). It may also convert 1,3,4,5,6-pentakisphosphate (InsP5) to PP-InsP4, affecting the growth suppressive and apoptotic activities of interferon-β in some ovarian cancers.

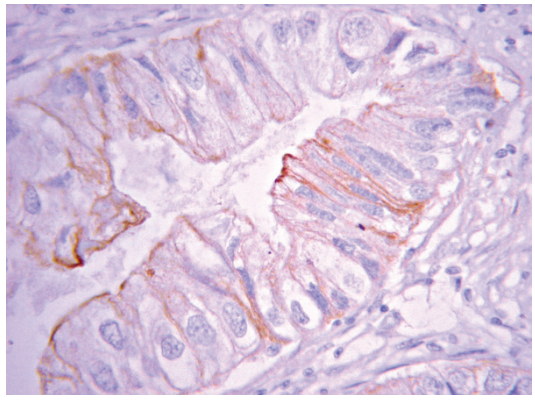


Western blot analysis of human testis lysate using Anti-IP6K2 (1 µg/mL), HRP goat Anti-mouse secondary antibody conjugate, and chemiluminescence detection revealed IP6K2, a roughly 50 kDa protein.

Anti-Phospholipase A2

(Catalogue No. 05-1406)

Phospholipases hydrolyze phosphoglycerides to form smaller compounds which often serve as critical second messengers. Phospholipase A2 (PLA2) hydrolyzes phosphatidylcholine to produce lysophosphatidylcholine, a second messenger involved in cell proliferation, adhesion, T-cell activation, and smooth muscle cell contractility. PLA2 isoforms include membrane-associated, Ca^{2+}-independent forms; cytosolic, Ca^{2+}-dependent forms; and secretory forms.

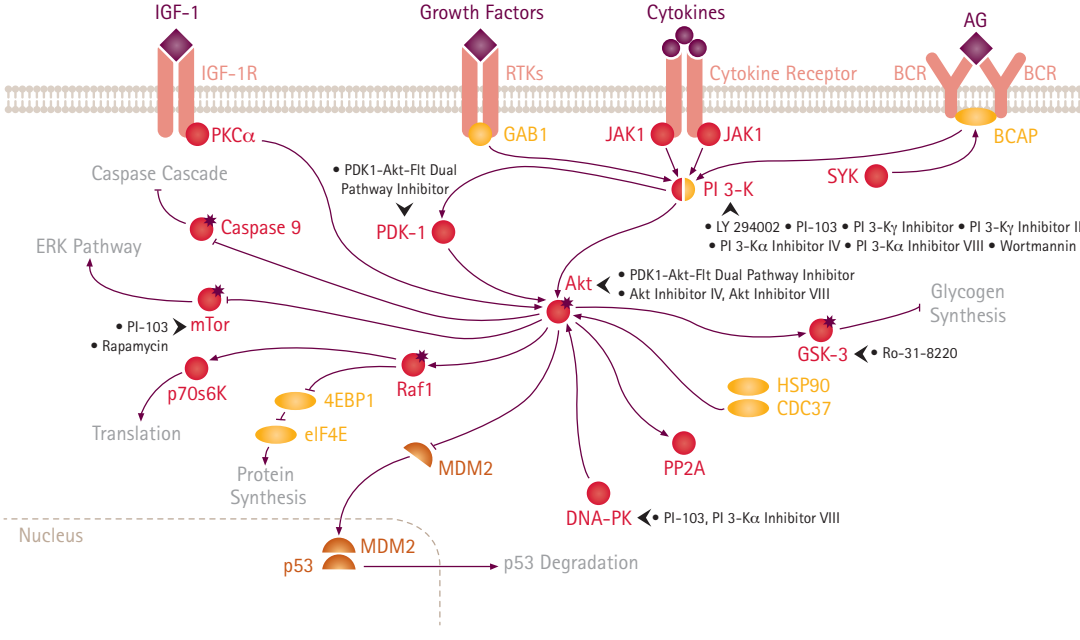


Cytoplasmic immunoreactivity detected in malignant cells in colon cancer tissue using Anti-PLA2 (05-1406) and IHC Select® detection with HRP-DAB.

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

(Catalogue No. 124031)

Activation of the PI 3-kinase/Akt/mTOR pathway stimulates cell proliferation and the translation process in response to nutrients and growth factors. Dysregulation of this pathway can lead to a variety of human tumors. A vast majority of solid tumors are reported to contain mutations either in PTEN or in the catalytic unit of their PI 3-K, resulting in increased enzymatic activity, cell proliferation, cell invasion, and metastasis. The InhibitorSelect™ PI 3-K/Akt/mTOR Signaling Pathway Inhibitor Panel enables multiparameter analysis, assessment of signal amplification/feedback, and comparison of biological effects of perturbing different parts of the pathway.



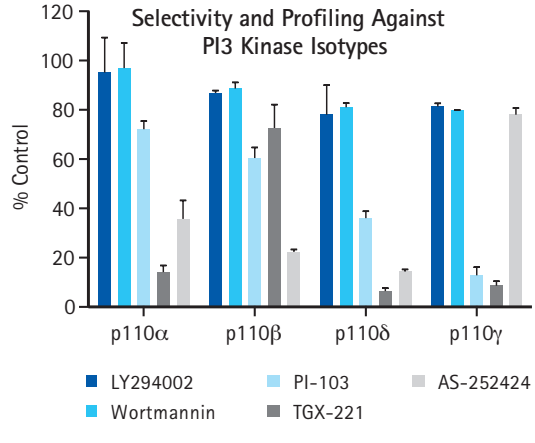
This panel consists of 12 highly potent, well-characterized, selective, and cell-permeable kinase inhibitors that target six proteins in the PI3 Kinase/Akt/mTOR pathway.

Phosphorylation
Ubiquitin
GTP
Calbiochem® Inhibitors

PI3 Kinase Activity/Inhibitor ELISA

(Catalogue No. 17-493)

The discovery of cancer-specific mutations in the gene coding for PI3K has transformed this field from an area of basic biochemistry into one of intense interest for target validation and drug development. These mutations single out class I PI3K as a particularly important contributor to oncogenesis. Most human cancers also show a gain of function in PI3K signaling. Easily evaluate PI3K activation and inhibition with this competitive ELISA kit.

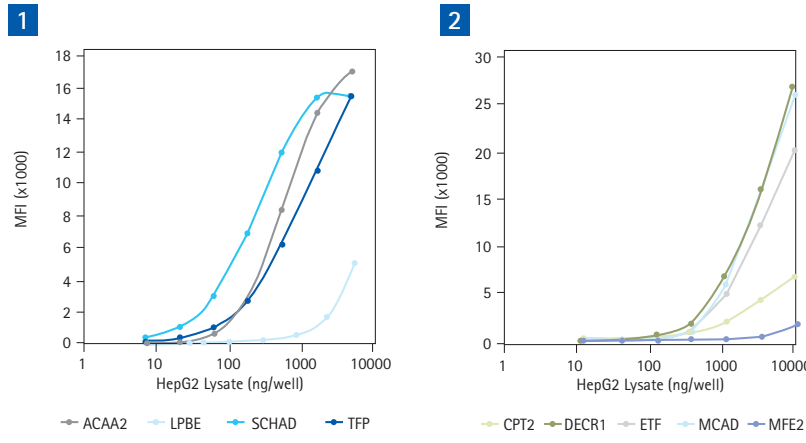


Differential inhibition of PI3 Kinase by isoform-specific versus general class I PI3 kinase inhibitors, as determined using the PI3 Kinase ELISA Kit.

MILLIPLEX® MAP Fatty Acid Oxidation (FAO) Panels 1 and 2

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

Monitoring the FAO pathway (especially the β-oxidation pathway) and any potential cellular metabolism changes in the human tissues and cells can reveal mechanisms of response to disease states, drug treatments, dietary changes or genetic mutations. The MILLIPLEX® MAP Human Fatty Acid Oxidation Panels include key enzymes that are involved in the β-oxidation pathway, and quantitation of these targets simultaneously in one reaction well (multiplexing) provides a more accurate snapshot of pathway activity.

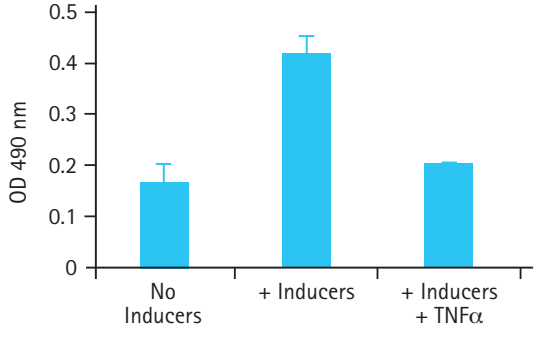


Multiplex analysis of human cell lysates and tissue extract with MILLIPLEX® MAP Human Fatty Acid Oxidation Magnetic Bead Panel 1 (left) and Panel 2 (right).

Adipogenesis Assay

(Catalogue No. ECM950)

Identifying regulators of adipogenesis, the formation of adipose tissue, may enable pharmacological prevention or reversal of obesity. This assay analyzes adipogenesis in the classic 3T3-L1 model. Commonly used inducers of adipogenesis, dexamethasone, IBMX and insulin, are included in convenient, ready-to-use formulations. Staining reagents provided enable quantitative characterization of adipogenesis induction or inhibition.



3T3-L1 cells induced for adipogenesis and then stained with Oil Red O demonstrated a significant increase in adipogenesis versus uninduced control cells. TNFα, a known inhibitor of adipogenesis, prevented differentiation of 3T3-L1 cells.

Key Products

Description	Catalogue No.
Antibodies	
Anti-DEPTOR	ABS222
Anti-phospho-Akt1 (Tyr326)	AB9927
Anti-PI3 Kinase, p110β	09-482
Anti-PI3 Kinase, p110γ, clone 17D7.2	05-1559
Anti-Pro-Insulin C-Peptide, clone C-PEP-01	05-1109
Small Molecule Inhibitors	
COX-2 Inhibitor II	236012
Fluvastatin, Sodium Salt	344095
Lovastatin, Sodium Salt	438186
Kits and Assays	
Human Apo AIV ELISA	EZHAPOA4-73K
MILLIPLEX® MAP Human Drug Metabolism Magnetic Bead Panel	HDMMAG-19K
MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	H0XPSMAG-16K
MILLIPLEX® MAP Rat/Mouse Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	RM0XPSMAG-17K
PI3 Kinase HTRF Screening Assay	33-016
PI3 Kinase HTS HTRF Assay	33-017

Nuclear Signaling

The nucleus, home to gene expression and regulation, is the endpoint of many signaling pathways, as cells respond to stimuli by altering gene expression patterns. Transcription factors, cell cycle checkpoint proteins, DNA damage detection and repair mechanisms are all key to nuclear signaling, gene expression and maintaining the integrity of the genome. Furthermore, gene expression is determined not only by hereditary information, coded in DNA sequence, but also by epigenetic marks, or modifications of DNA and associated proteins that are not necessarily inherited. Epigenetic mechanisms include modifications to histones, methylation of DNA, remodeling of chromatin, and signaling via noncoding RNA molecules. While the genome remains relatively static, the complementary epigenome adapts to environmental influences and can confer unique characteristics ad phenotypes to different cell types, tissues, and organisms. Today's biomedical researchers understand that epigenetic marks are as important as DNA sequence in completing the cell's complex signaling network.

Transcription Factors

Transcription factors bind specific DNA sequences and control transcription of adjacent genes. These signaling proteins cooperate with a number of other proteins, such as coactivators, chromatin remodelers, histone acetylases, deacetylases, kinases, and methylases, to promote or block the recruitment of RNA polymerase to specific genes. Regulation of transcription factors by upstream modifiers ensure exact spatial and temporal expression of genes. Transcription factors are activated sequentially and they may also recruit coregulators, coactivators, or corepressors.

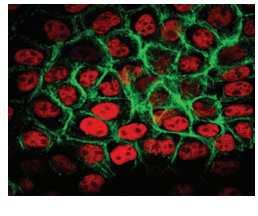
Featured Products

Anti-p53 (pantropic), clone DO-1

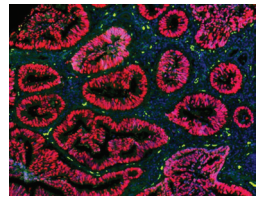
(Catalogue No. MABE327)

p53 is a ubiquitous transcription factor that is most widely known for its function as a tumor suppressor, through its regulation of cell growth and survival. p53 is activated in response to cell stress such as DNA damage and induces either cell cycle arrest or apoptosis. It coordinates the expression of multiple genes including p21, Bax, and Puma. p53 undergoes phosphorylation and acetylation, which may regulate its activity. It may also be ubiquitinated via the Akt-MDM2 pathway, resulting in degradation by the 20S proteasome. Mutations in p53 are prevalent in many types of cancers, underscoring the therapeutic relevance of this protein.

A



B

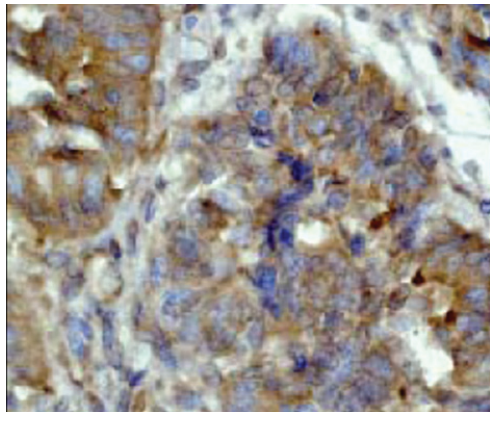


Nuclear expression of p53 in A431 cells (A) as well as human colorectal cancer cells (B) shown by immunocytochemical staining using Anti-p53 (pantropic), clone DO-1. Staining was visualized with a Donkey Anti-Mouse IgG conjugated to a red fluorescent dye. Actin has been labeled with AlexaFluor® 488 dye - Phalloidin (Green).

Anti-SMAD2

(Catalogue No. 04-1029)

SMAD2 is one of the TGFβ receptor-regulated SMAD transcription factors. When SMAD2 is phosphorylated upon binding of extracellular TGFβ/activin to receptors, SMAD2 activates transcription of self-renewal genes, such as Nanog. Interestingly, under different cellular conditions, Wnt signaling cooperates with SMAD2 to activate differentiation genes. SMAD2 therefore is a central switch in signaling pathways that determine cell fate.

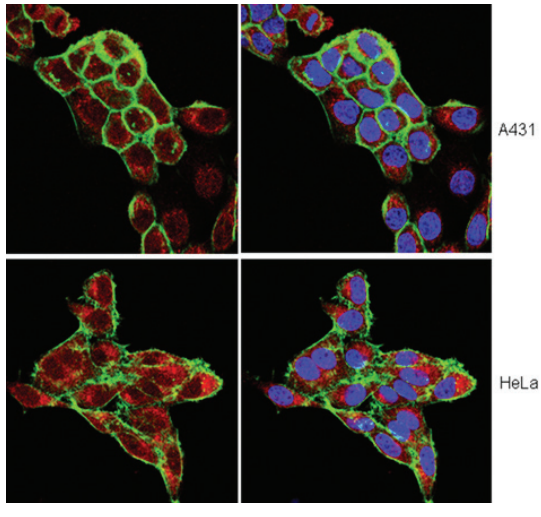


Smad2 is expressed in tumors, in which cells are actively undergoing both self-renewal and differentiation. Immunohistochemical staining of paraffin-embedded human adenocarcinoma of uterus using Anti-Smad2 (Cat. No. 04-1029).

Anti-FOXO1

(Catalogue No. 05-1075)

Forkhead box O (FOXO) transcription factors modulate metabolic functions. Given the relatively high expression of FOXO1 in insulin-responsive tissues, this transcription factor is poised to regulate energy metabolism. When nutrient and insulin levels are low, FOXO1 promotes expression of gluconeogenic enzymes. Conversely, in the fed state, insulin levels rise, stimulating glucose uptake and inhibiting FOXO1 activation of gluconeogenesis. Under certain pathophysiologic conditions, including insulin resistance, negative signaling to FOXO1 is compromised.

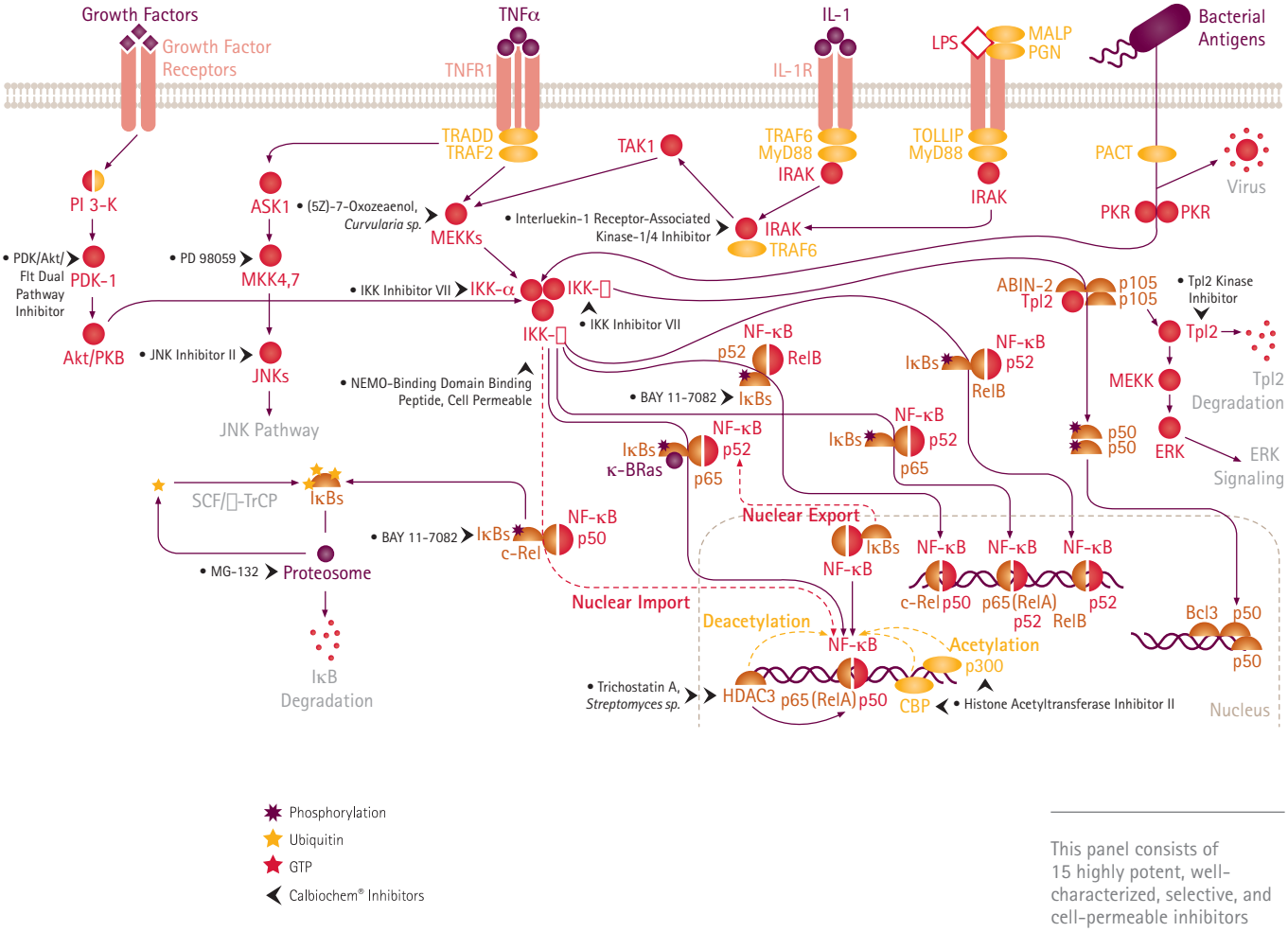


FOXO1 localizes to the nuclei of A431 and HeLa cells as determined using confocal fluorescent immunocytochemistry with Anti-FOXO1 (red, Cat. No. 05-1075). Also shown are actin (green) and nuclei (blue).

InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel

(Catalogue No. 481487)

Nuclear factor κB (NF-κB) regulates genes involved in inflammation, autoimmune response, cell proliferation, and apoptosis. The Rel/NF-κB signal transduction pathway is dysregulated in a variety of human cancers, especially those of lymphoid cell origin. Designing antitumor agents to block NF-κB activity or to increase its sensitivity to conventional chemotherapy may have great therapeutic value. Basing such target validation studies on a set of structurally diverse molecules such as in the InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel can provide a powerful starting point for drug discovery.



This panel consists of 15 highly potent, well-characterized, selective, and cell-permeable inhibitors that target 14 proteins in the NFκB pathway.

Key Products

Description	Catalogue No.
Antibodies	
Anti-ATR	09-070
Anti-FOXO1	05-1075
Anti-p53 (pantropic), clone DO-1	MABE327
Anti-SMAD2	04-1029
Small Molecule Inhibitors	
InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel	481487
JAK Inhibitor I	420099
STAT3 Inhibitor III, WP1066	573097
STAT3 Inhibitor Peptide, cell-permeable	573096
Kits and Assays	
FlowCellect® Multi-STAT Activation Profiling Kit	FCCS025550
MILLIPLEX® MAP ATF2 (Thr69/71) MAPmate	46-658
MILLIPLEX® MAP STAT1 (Tyr701) MAPmate	46-655
MILLIPLEX® MAP STAT3 (Ser727) MAPmate	46-624
MILLIPLEX® MAP STAT 5-Plex Panel	48-610MAG
Universal EZ-TFA™ Transcription Factor Assay, Colorimetric	70-501

Measurement of Immune Cell Function Using Amnis® Imaging Flow Cytometry

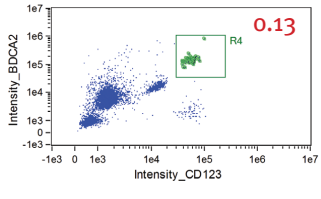
Immune cells sense their environment by either binding and processing soluble mediators of inflammation or through direct contact with other cells, resulting in signal transduction and activation or suppression of effect or function. Cell signaling is most completely assessed through quantitative imaging assays, but immune cells present significant challenges to image-based analysis due to their rarity and the need for simultaneous multispectral immunophenotyping, making statistically robust quantification difficult. Cell signaling assays are ideally performed by using the Amnis ImageStream® and FlowSight® imaging flow cytometry platforms, which quantify imagery of large populations of cells.

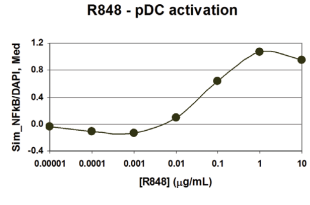
Application Example

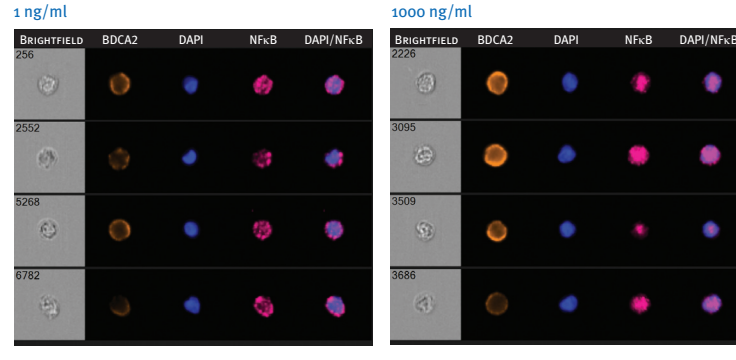
Measurement of NF-κB activation in whole blood plasmacytoid dendritic cells (pDC)

Molecular translocation of transcription factors from the cytoplasm to the nucleus is a pivotal event in many processes critical to cellular activation, differentiation, and host defense. Nuclear translocation events within non-adherent immune cells can be quantified by correlating transcription factor and nuclear images collected in flow using the Amnis ImageStream®X Mark II and FlowSight® imaging flow cytometers. The results demonstrate the unique suitability of the Amnis® imaging cytometers to cell signaling assays.

As shown below, pDC (a subpopulation of innate immune cells) express pattern recognition receptors that transmit activating signals upon ligand binding. Translocation of NF-κB was measured as a marker for TLR7-induced activation in whole blood pDC. NF-κB translocation was measured using cross-correlation analysis of nuclear and NF-κB images from each cell in the gated pDC population from whole blood samples exposed to a range of R848 doses[1]. Images of representative cells from the 1 ng/mL (left) and the 1000 ng/mL (right) samples are shown.







1. This novel, quantitative method of measuring translocation has been published: George TC et al. Quantitative measurement of nuclear translocation events using similarity analysis of multispectral cellular images obtained in flow.J Immunol Methods. 2006 Apr 20;311(1-2):117-29.

Access the complete library of Amnis application notes and other resources at www.amnis.com.

Cell Cycle and DNA Damage

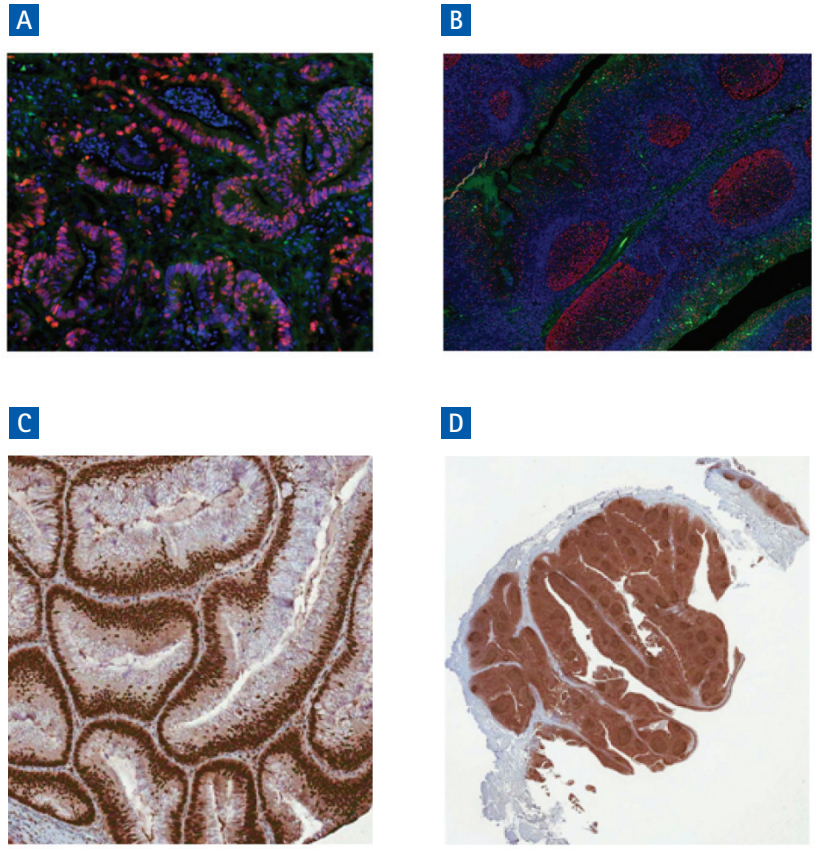
In all multicellular organisms, proliferating cells decide either to enter the cell cycle or reach a quiescent state. Quiescent cells must also decide whether to stay in a non-proliferating state or re-enter the cycle. Cell cycle, or the process of cell growth and duplication, is the regulatory point for proliferation and development of multicellular organisms. Nuclear signaling controls most checkpoints of the cell cycle, which in turn are regulated by chromatin structure. Chromosomal instability is a hallmark of many cancers, and is seen as either a cause or a symptom of the unchecked proliferation exhibited by tumor cells. Studying the mechanisms by which cells control changes in DNA structure and respond to DNA damage will help to elucidate the factors that cause aging, cellular degeneration, cancer, and death.

Featured Products

Anti-PCNA, clone PC10

(Catalogue No. MABE288)

Proliferating cell nuclear antigen (PCNA) functions as a clamp on replicating DNA, increasing processivity of leading strand synthesis. In cases of DNA damage and failure of the DNA repair pathways, PCNA mediates translesion DNA synthesis by recruiting low-fidelity DNA polymerases, allowing replication to progress in spite of damage caused by replication stress, ionizing radiation and other factors.



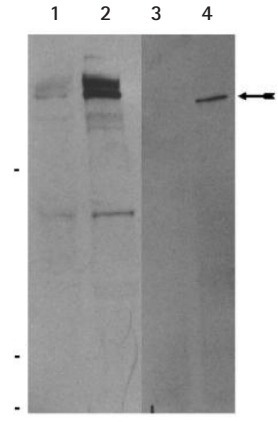
Nuclear localization of PCNA was observed in human colorectal adenocarcinoma tissues and in lymphocytes of human tonsil tissues using immunofluorecence and immunohistochemistry. Immunofluorescent analysis of human colon adenocarcinoma tissue (A) and human tonsil tissue (B) were performed using 1:500 Anti-PCNA, clone PC10 (red). Also labeled were actin (green) and nuclei (blue). Immunohistochemistry analysis of human colon adenocarcinoma tissue (C) and human tonsil tissue (D) were performed using 1:1,000 Anti-PCNA, clone PC10. Reactivity was detected using an Anti-mouse secondary antibody and HRP-DAB.

Featured Products

Anti-phospho-ATM (Ser1981)

(Catalogue No. 05-740)

Ataxia telangiectasia mutated kinase (ATM) regulate cell cycle checkpoints and DNA repair. In unirradiated cells, ATM is present as an inactive homodimer or multimer. Double-stranded breaks in DNA caused by ionizing radiation cause rapid ATM kinase activation through dissociation of this complex and ATM autophosphorylation at Ser1981. Phosphorylated ATM in turn activates diverse effector proteins, such as Chk2, BRCA1 and MDM2.

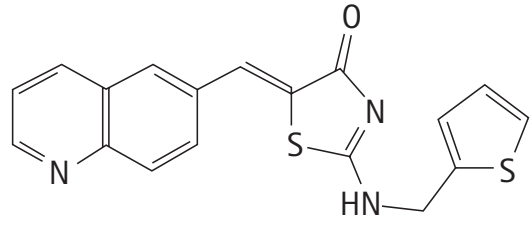


ATM is phosphorylated in response to radiation-induced DNA damage. Western blot analysis of crude HeLa cell extracts (lanes 1 & 2) or ATM-containing immune complexes (lanes 3 & 4) from either untreated (lanes 1 & 3) or γ-irradiated (lanes 2 & 4) probed with 0.5 µg/mL of Anti-phospho-ATM (Ser1981, Cat. No. 05-740).

Cdk1 Inhibitor IV, RO-3306

(Catalogue No. 217699)

Using specific inhibitors of cyclin-dependent kinases (Cdks) is an efficient way to study cell cycle progression and identify Cdk substrates, which are determined by which cyclin protein is bound. G1/S cyclin-bound Cdk1 prepares the cell for entry into S phase. After this step, cyclins 1 and 2 are replaced by S phase cyclins in the Cdk1 complex, which lead to DNA replication. Binding of M phase cyclins to Cdk1 promotes spindle formation, and ultimately, Cdk1 activates the anaphase promoting complex to help complete mitosis.

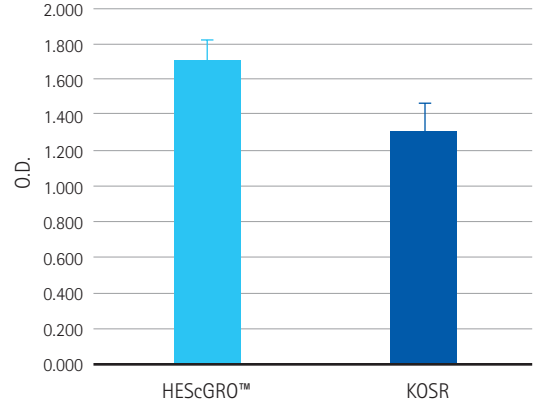


This cell-permeable quinolinyl thiazolinone compound is a potent and ATP-competitive inhibitor of Cdk1 (Ki = 35 nM and 110 nM for Cdk1/B1 and Cdk1/A, respectively). Short-term treatment results in fully reversible G2/M cell cycle arrest, while prolonged treatment results in apoptosis in proliferating cancer cells, but not in nontumorigenic epithelial cell lines.

BrdU Cell Proliferation Kit

(Catalogue No. 2750)

This non-isotopic, colorimetric assay enables *in vitro* quantitation of newly synthesized DNA of actively proliferating cells. Bromodeoxyuridine (BrdU) is incorporated into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, BrdU is detected immunochemically allowing the assessment of the population of cells which are synthesizing DNA.

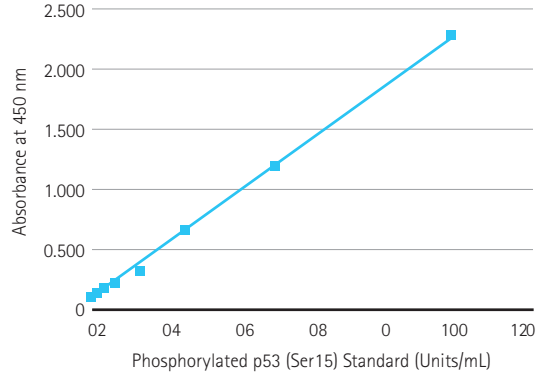


The BrdU cell proliferation kit (Cat. No. 2750) was used to measure proliferation of H9 human embryonic stem cells in HEScGRO™ and KOSR media, after cells were enzymatically expanded for 12 passages. Increased BrdU incorporation indicated faster cell proliferation in HEScGRO™ medium.

Phospho-p53(Ser15) STAR ELISA Kit

(Catalogue No. 17-475)

In response to DNA damage, p53 is phosphorylated on serine 15 by ATM and ATR kinases, inducing further p53 phosphorylation and activation. Activated p53 induces gene expression, such as for the Cdk inhibitor p21, which, in cooperation with p19ARF, causes cell cycle arrest. Inactivation or loss of p53 is associated with deregulation of the cell cycle and DNA replication, inefficient DNA repair, and tumorigenesis. The phospho-p53(Ser15) STAR (Signal Transduction Assay Reaction) ELISA is a fast, sensitive method to detect activated p53.



Typical phospho-p53(Ser15) Standard Curve. 100 µL of progressive 2-fold dilutions of the p53 standard included in the kit were analyzed as described in the assay instructions.

Key Products

Description	Catalogue No.
Antibodies	
Anti-ATM	07-1286
Anti-phospho-ATM (Ser1981)	05-740
Anti-phospho-Chk2 (Thr68), clone E126, rabbit monoclonal	04-1471
Cell Cycle-S Phase Pathway Explorer Antibody Minipack	15-119
Cell Cycle-G2/M Phase Pathway Explorer Antibody Minipack	15-120
Anti-Chk1	04-207
Anti-Plk1	05-844
Anti-Wee1	06-972
Small Molecule Inhibitors	
Cdk1 Inhibitor IV, RO-3306	217699
Roscovitine	557360
Chk2 Inhibitor II	220486
Kits and Assays	
BrdU Cell Proliferation Kit	2750
FlowCellect® Bivariate Cell Cycle Kit for G2/M Flow Cytometry Analysis	FCCH025103
Phospho-p53 (Ser15) STAR ELISA Kit	17-475

Histone and Other Modifications

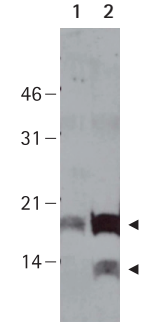
The most commonly studied and best understood histone modifications are acetylation, phosphorylation, methylation, and ubiquitination. Histone modifications regulate DNA transcription, repair, recombination, and replication, and can alter local chromatin architecture.

Featured Products

Anti-acetyl-Lysine

(Catalogue No. 05-515)

Acetylation of lysine residues within proteins has emerged as an important mechanism used by cells to reverse gene silencing. The acetylation of non-histone proteins, such as transcription factors, as well as histone acetylation appears to be involved in this process and may result in structural transitions and specific signaling within discrete chromatin domains.

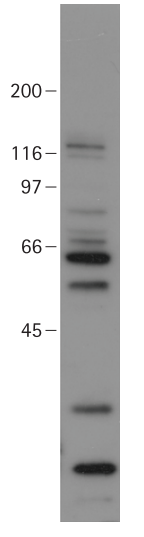


Western blot analysis of sodium butyrate-treated (lane 2) and untreated (lane 1) HeLa cells post-acid extraction using Anti-acetyl-Lysine (Cat. No. 05-515).

Anti-dimethyl-Arginine, asymmetric (ASYM24)

(Catalogue No. 07-414)

Arginine methylation is a common post-translational modification that is catalyzed by the protein arginine methyltransferase (PRMT) family of enzymes. Asymmetrical omega-NG, NG-dimethylated arginines (ADMAs) have been shown to inhibit nitric oxide (NO) synthase, thereby regulating blood vessel constriction and consequently cardiovascular disease, diabetes, hypertension and other disorders of the endothelium.

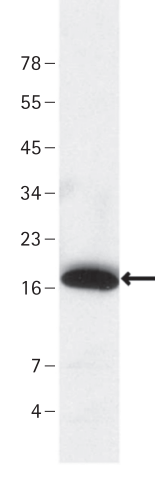


Western blot analysis of Jurkat cell lysate probed with Anti-dimethyl-Arginine, asymmetric (ASYM24), (1:500). Several proteins containing asymmetrically dimethylated arginines were detected, including p110, p75 and Sam68.

Anti-dimethyl Histone H3 Lys4

(Catalogue No. 07-030)

Anti-dimethyl histone H3 Lys4 is just one example of a histone modification-specific antibody. Merck Millipore offers over 200 validated antibodies, recombinant proteins, and kits to analyze histone modifications. H3 Lys4 is methylated by the Set1 enzyme, with regulation by Ctk1 kinase, to establish euchromatin boundaries. While H3 Lys4 trimethylation is associated with transcriptional initiation, the function of the dimethylated form may be gene- and context-specific.

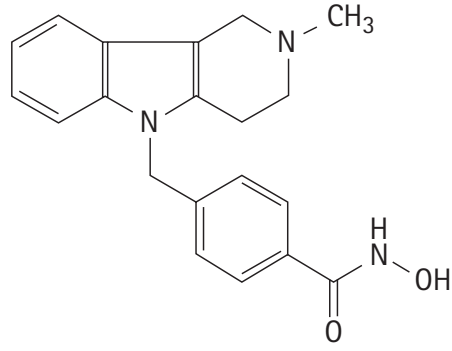


Western blot analysis of Hela cell lysate acid precipitate probed with Anti-dimethyl Histone H3 (Lys4).

HDAC Inhibitor XXIII, Tubastatin A

(Catalogue No. 382187)

Histone deacetylases (HDACs) are key regulators of gene expression and function. HDACs remove acetyl groups, leading to decreased gene expression. Because hyperacetylation has been associated with tumorigenesis, HDAC inhibitors are an emerging class of candidates for cancer therapy.

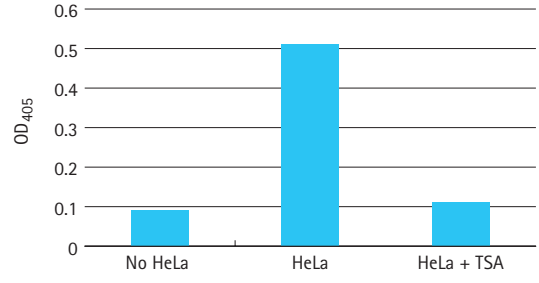


A cell-permeable carbazolohydroxamate that acts as a highly potent HDAC6-selective inhibitor (IC_{50} = 15 nM). Prevents neuronal cell death (by ≥95% at 10 µM) upon oxidative stress induction by HCA and selectively induces cellular α-tubulin, but not histone H4, hyperacetylation (2.5 to 5 µM) in primary rat cortical neuron cultures.

HDAC Assay Kit, Colorimetric Detection

(Catalogue No. 17-374)

Because lysine acetylation of histones often creates open chromatin structure permitting transcription, HDACs usually cause decreased gene expression. The colorimetric HDAC assay kit is a simple, two-step procedure designed for use in 96- or 384- multiwell plates. Alternatively, use our HDAC assay kits supporting fluorometric detection or radiometric detection.

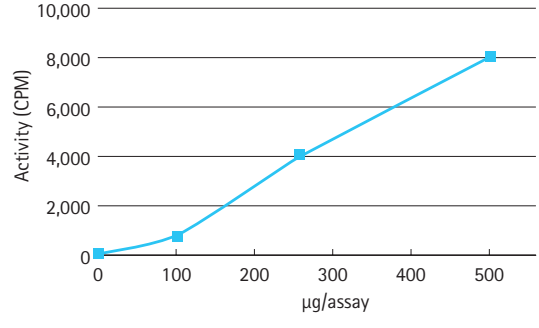


Inhibition of histone acetylation by trichostatin A (TSA). HeLa Nuclear Extract in the absence or presence of 1 µM TSA was assayed with the colorimetric HDAC Assay Kit (Cat. No. 17-374). Absorbance was detected at 405 nm.

Histone Methyltransferase Assay

(Catalogue No. 17-330)

Elucidate the mechanism by which histone methyltransferases are regulated using this robust radiometric assay. Although the functions of histone methylation marks are rapidly being discovered, less is known about the regulation of methyltransferases, such as EZH2 and the Set proteins.

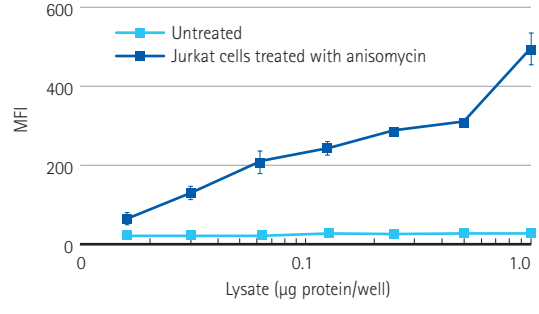


The methyltransferase PRMT1 (Cat. No. 14-474) methylates core histones *in vitro*, as measured using the Histone Methyltransferase Assay Kit (17-330). Assay background was subtracted from the actual counts to yield the results.

MILLIPLEX® MAP H2A.X Phosphorylation Assay Kit MAPmates™

(Catalogue No. 46-692)

Phosphorylation of histones commonly occurs during chromosome condensation in mitosis, and during apoptosis. Phosphorylation of H2A.X recruits the repair complex at the site of DNA damage, and is a marker for double strand breaks. The MILLIPLEX® MAP phosphohistone H2A.X (Ser139) MAPmates™ are ideal for bead-based measurement of phosphorylated histone H2A.X (Ser139) in cell lysates using Luminex® technology.



MILLIPLEX® MAP detection of changes in phosphorylation of histone H2A.X (Ser139) in Jurkat cells stimulated with or without 25 mM anisomycin. The Median Fluorescent Intensity (MFI) was measured using the Luminex 200™ instrument.

Key Products

Description	Catalogue No.
Antibodies	
Anti-acetyl-Lysine	05-515
Anti-dimethyl-Arginine, asymmetric (ASYM24)	07-414
Anti-dimethyl-Histone H3 (Lys27)	07-452
Anti-dimethyl-Histone H3 (Lys4)	07-030
Anti-dimethyl-Histone H4 (Lys20)	07-1584
Anti-monomethyl-Histone H3 (Lys27)	07-448
Anti-monomethyl-Histone H3 (Lys4)	07-436
Anti-monomethyl-Histone H4 (Lys20), clone NL314	04-735
Anti-trimethyl-Histone H3 (Lys27)	07-449
Anti-trimethyl-Histone H3 (Lys4)	05-745R
Anti-trimethyl-Histone H4 (Lys20)	07-463
Anti-ubiquityl-Histone H2A, clone E6C5	05-678
Small Molecule Inhibitors	
HDAC Inhibitor XXII, Tubastatin A	382187
Hdm2 E3 Ligase Inhibitor	373225
Histone Acetyltransferase p300 Inhibitor, C646	382113
LSD1 Inhibitor	489476
LSD1 Inhibitor II, S2101	489477
Histone Lysine Methyltransferase Inhibitor	382190
Histone Deacetylase Inhibitor VII, 106	382173
HMTase Inhibitor V, UNC0224	382193
I-BET	401010
Kits and Assays	
FlowCellect® DNA Damage Histone H2A.X Dual Detection Kit	FCCS025153
HAT Assay Kit	17-289
HDAC Assay Kit, colorimetric detection	17-374
Histone Methyltransferase Assay	17-330
MILLIPLEX® MAP H2A.X Phosphorylation Assay Kit MAPmates™	46-692
SIRTainty™ Class III HDAC Assay	17-10090
Proteins	
HDAC1 Active recombinant protein	14-838
HDAC4 Active recombinant protein	14-828
HDAC7 Active recombinant protein	14-832
UbcH2 Conjugating Enzyme	14-807
Ubiquitin Activating Enzyme E1	14-857

DNA Methylation

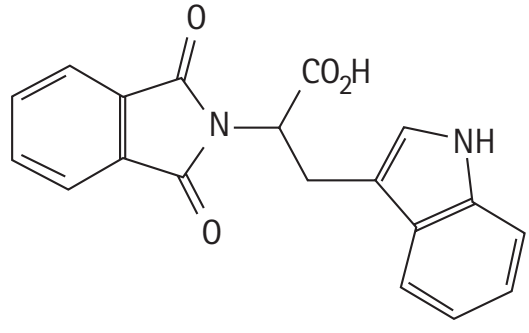
DNA methylation, involving the addition of methyl to the 5 carbon position of cytosine residues in CpG islands, is catalyzed by DNA cytosine-5-methyltransferases. About 1% of the genome consists of 500-2000 bp CpG-rich areas or islands. About half of all CpG islands correspond to transcription start sites and promoters of expressed genes. Methylation of cytosine residues results in the formation of 5-methylcytosine (5mC). 5-methylcytosines can also be hydroxylated to form 5-hydroxymethylcytosines (5-hmC). 5-mC and 5-hmC modifications are regulated by a number of nuclear enzymes and signaling molecules. These modifications play important roles in gene regulation underlying normal biological function, as well as in the development of diseases such as cancer.

Featured Products

DNA Methyltransferase Inhibitor, RG108

(Catalogue No. 260920)

Methylation of DNA is an important gene regulation mechanism, especially for epigenetic silencing of genes to achieve terminal differentiation. Using DNA methyltransferase inhibitors to reverse epigenetic silencing can be a useful tool for modulating cell fate. However, many methyltransferase inhibitors exhibit considerable cytotoxicity. RG108, an anti-proliferative but not cytotoxic inhibitor, has the potential to help elucidate the mechanisms and tissue specificity of cell fate determination by DNA methylation.



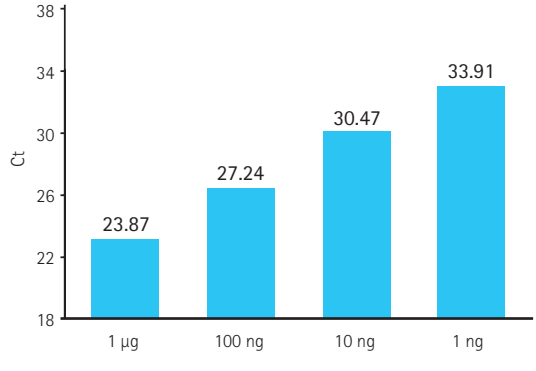
RG108 is a cell-permeable, specific DNA methyltransferase inhibitor (IC_{50} = 115 nM for CpG methylase M.SssI). Unlike the commonly used inhibitor 5-azacytidine, RG108 directly interacts with DNA methyltransferase active site via its carboxy group.

CpGenome™ Turbo Bisulfite Modification Kit

(Catalogue No. S7847)

The CpGenome™ Turbo Bisulfite Modification Kit is designed to simplify and streamline the bisulfite modification process. This kit contains all key reagents for bisulfite modification to enable you to go from input sample to recovery of modified DNA in 90 minutes, with conversion efficiencies of 99.9%. Effective with as little as 1 ng input DNA, the kit allows for modified DNA recovery in as little as 25 microliters.

Reliable Performance Across a Range of Input Sample Amounts

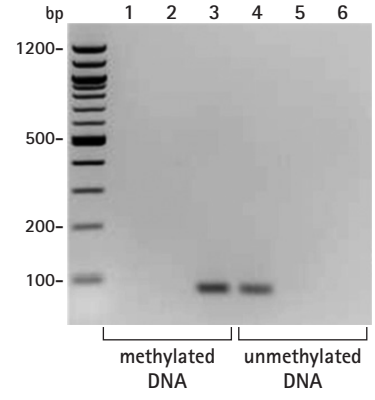


Sensitive and Reliable Bisulfite Conversion from 1 ng to 1 µg Methylated DNA (Cat. No. S7821) was bisulfite treated as described in the CpGenome™ Turbo protocol and eluted in 50 µL (1 µg and 100 ng samples) or in 25 µL (10 ng and 1 ng samples). Conversion was evaluated by quantitative PCR using the CpG WIZ® MGMT methylated primer set (Cat. No. S7803).

CpGenome™ Human Methylated DNA CpGenome™ Human Non-Methylated DNA

(Catalogue Nos. S8001M and S8001U)

Facilitate interpretation of DNA methylation analyses using this complete set of positive and negative control DNA. The CpGenome™ unmethylated DNA standard is purified from double knockout HCT116 DKO(-DNMT1 and -DNMT3b), resulting in less than 5% methylated CpGs. The CpGenome™ Human Methylated DNA Standard has been enzymatically methylated at at least 95% of CpG dinucleotides by M.SssI methyltransferase 2. These standards are fully compatible with CpGenome™ Turbo Bisfulfite Conversion Kit and CpG WIZ® DNA Amplification Kits.



Human methylated and non-methylated DNA was bisulfite-modified using the CpGenome™ Turbo Bisulfite Kit (Cat. No. S7847) and amplified with the CpG WIZ® BRCA1 Amplification Kit (Cat. No. S7830).

Primer specificity:

Lane 1,4: Unmethylated, bisulfite mod.

Lane 2,5: Wildtype, no bisulfite

Lane 3,6: Methylated, bisulfite mod.

Key Products

Description	Catalogue No.
Antibodies	
Anti-5-Hydroxymethylcytosine, clone AB3/63.3	MABE176
Anti-5-Methylcytosine, clone use mAb (162 33 D3)	NA81-50UG
Anti-5-Methylcytosine, clone 33D3	MABE146
Anti-acetyl-MeCP2 (Lys464)	ABE28
Anti-CBX-4, clone 10H10.2	MAB11012
Anti-DNA Methyltransferase 1	AB3429
Anti-DNMT3A2	07-2050
Anti-Kaiso (659-672) Goat pAb	PC723-100UG
Anti-MBD1 (methyl-CpG binding domain) protein 1	09-833
Anti-MBD1, C-terminus	07-2054
Anti-MeCP2 (Rabbit Polyclonal)	07-013
Anti-Methylcytosine Dioxygenase TET1	09-872
Anti-phospho-DNMT1(Ser714)	07-1594
Small Molecule Inhibitors	
DNA Methyltransferase Inhibitor II, SGI-1027	260921
Kits and Assays	
CpG MethylQuest™ DNA Isolation Kit	17-10035
CpG WIZ® BRCA1 Amplification Kit (human)	S7830
CpG WIZ® Fragile X Amplification Kit (human)	S7807
CpG WIZ® MGMT Amplification Kit (human)	S7803
CpG WIZ® Oct-4 Amplification Kit (mouse)	S7840
CpG WIZ® p14/ARF Amplification Kit (human)	S7817
CpGenome™ 5mC and 5hmC DNA Standard Set	S8005
CpGenome™ Universal Methylated Mouse DNA Standard	S8000
Proteins	
CpG MethylQuest™ Protein	14-921

Chromatin Interaction

Gene expression is regulated by the interaction of various proteins to chromatin. Some of the most powerful investigations into epigenetic mechanisms of disease have utilized chromatin immunoprecipitation (ChIP). ChIP can detect and relatively quantify specific protein-DNA and protein-protein interactions within cells or tissue, at a single locus or multiple loci.

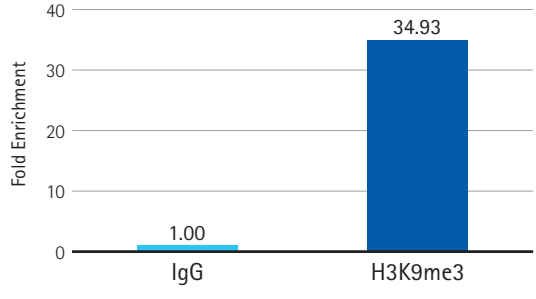
At the most elemental level, ChIP involves chemically crosslinking proteins to DNA sequences, which is followed by immunoprecipitation of the crosslinked complex, and final analysis of the resultant DNA by endpoint or quantitative PCR (qPCR), microarrays, or next-generation sequencing.

Featured Products

ChIPAb+™ Trimethyl-Histone H3 (Lys9)

(Catalogue No. 17-625)

Antibody recognition in the context of chromatin can differ from other immunoassays. Merck Millipore's extensive line of ChIPAb+™ antibody/primer kits provide a ChIP-validated antibody, each lot individually validated and tested for ChIP, a negative control antibody, plus control primers for amplifying a known enriched locus, to help you validate your results. The ChIPAb+™ Trimethyl-Histone H3 (Lys9) set enables reliable studies of this canonical repressive epigenetic mark. The kit includes the Anti-trimethyl-Histone H3 (Lys9) antibody, a negative control antibody (purified rabbit IgG), and qPCR primers which amplify a 117 bp region within the 3' end of the human ZNF554 gene.

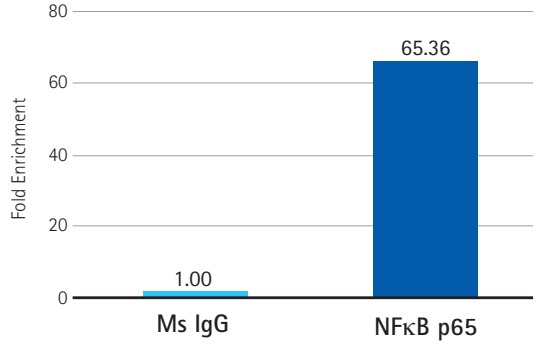


ChIPAb+™ Trimethyl-histone H3 (Lys9) (Cat. No. 17-625): Sonicated chromatin from NIH 3T3 L1 cells was subjected to chromatin immunoprecipitation using either normal rabbit IgG or Anti-trimethyl-histone H3 (Lys9) antibody and the Magna ChIP™ A Kit (Cat. No. 17-610). Successful enrichment of trimethyl-histone H3 (Lys9)-associated DNA fragments was verified by qPCR using primers flanking the mouse p16 promoter.

EZ Magna ChIP™ A/G Chromatin Immunoprecipitation Kit

(Catalogue No. 17-10086)

A blend of protein A+G beads in this kit allows ChIP using a broader range of antibodies than protein A or G alone. The magnetic bead-based rapid protocol enables performance of ChIP in as little as 1 day. The kit also includes spin columns to make DNA purification easier and more reliable. Additionally, the kit is compatible with ChIPAb+™ validated antibody and primer sets.



Specific localization of NFκB binding via one-day ChIP using the EZ-Magna ChIP™ kit. Sonicated chromatin prepared from serum-starved, TNFα-treated HEK293 cells (~3 x 10^6 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 4 µg of either Normal Mouse IgG, or 4 µg Anti-NFκB p65 (RelA) (components contained in NFκB p65 ChIPAb+™ kit (Catalogue No. 17-10060).

Immunoprecipitation of NFκB p65 (RelA)-associated DNA fragments was verified by qPCR using primers directed against IκBα.

Key Products

Description	Catalogue No.
Antibodies	
ChIPAb+™ Histone H2A.Z	17-10048
ChIPAb+™ Histone H2B	17-10054
ChIPAb+™ Histone H3 (C-term)	17-10046
ChIPAb+™ Histone H3 (Unmod Lys4)	17-675
ChIPAb+™ Acetyl Histone H3	17-615
ChIPAb+™ Acetyl-Histone H3 (Lys4)	17-10050
ChIPAb+™ Acetyl-Histone H3 (Lys9)	17-658
ChIPAb+™ Acetyl-Histone H3 (Lys14)	17-10051
ChIPAb+™ Monomethyl Histone H3 (Lys27)	17-643
ChIPAb+™ Dimethyl-Histone H3 (Lys4)	17-677
ChIPAb+™ Dimethyl-Histone H3 (Lys9)	17-648
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-614
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-678
ChIPAb+™ Trimethyl-Histone H3 (Lys9)	17-625
ChIPAb+™ Trimethyl-Histone H3 (Lys27)	17-622
ChIPAb+™ Trimethyl-Histone H3 (Lys36)	17-10032
ChIPAb+™ Trimethyl-Histone H3 (Lys79)	17-10130
ChIPAb+™ Phospho-Histone H3 (Ser10)	17-685
ChIPAb+™ Acetyl Histone H4	17-630
ChIPAb+™ Acetyl-Histone H4 (Lys5)	17-10045
ChIPAb+™ CREB	17-600
ChIPAb+™ CTCF	17-10044
ChIPAb+™ EED	17-663
ChIPAb+™ EED (Rabbit Polyclonal)	17-10034
ChIPAb+™ ERα	17-603
ChIPAb+™ EZH2, clone AC22	17-662
ChIPAb+™ HDAC1	17-608
ChIPAb+™ p53	17-613
ChIPAb+™ Phospho-CREB (Ser133)	17-10131
ChIPAb+™ REST	17-641
ChIPAb+™ RNA Polymerase II	17-620
ChIPAb+™ SMRT	17-10057
ChIPAb+™ Sox-2, clone 6F1.2	17-656
ChIPAb+™ Sp1	17-601
ChIPAb+™ SUZ12	17-661
ChIPAb+™ TATA Binding Protein (TBP)	17-10098
Kits and Assays	
EZ Magna ChIP™ A/G Chromatin Immunoprecipitation Kits	17-10086
Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Preparation Kit	17-1010
Magna ChIP™ A/G Chromatin Immunoprecipitation Kits	17-10085
Magna ChIP™ HT96 Chromatin Immunoprecipitation Kit	17-10077

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