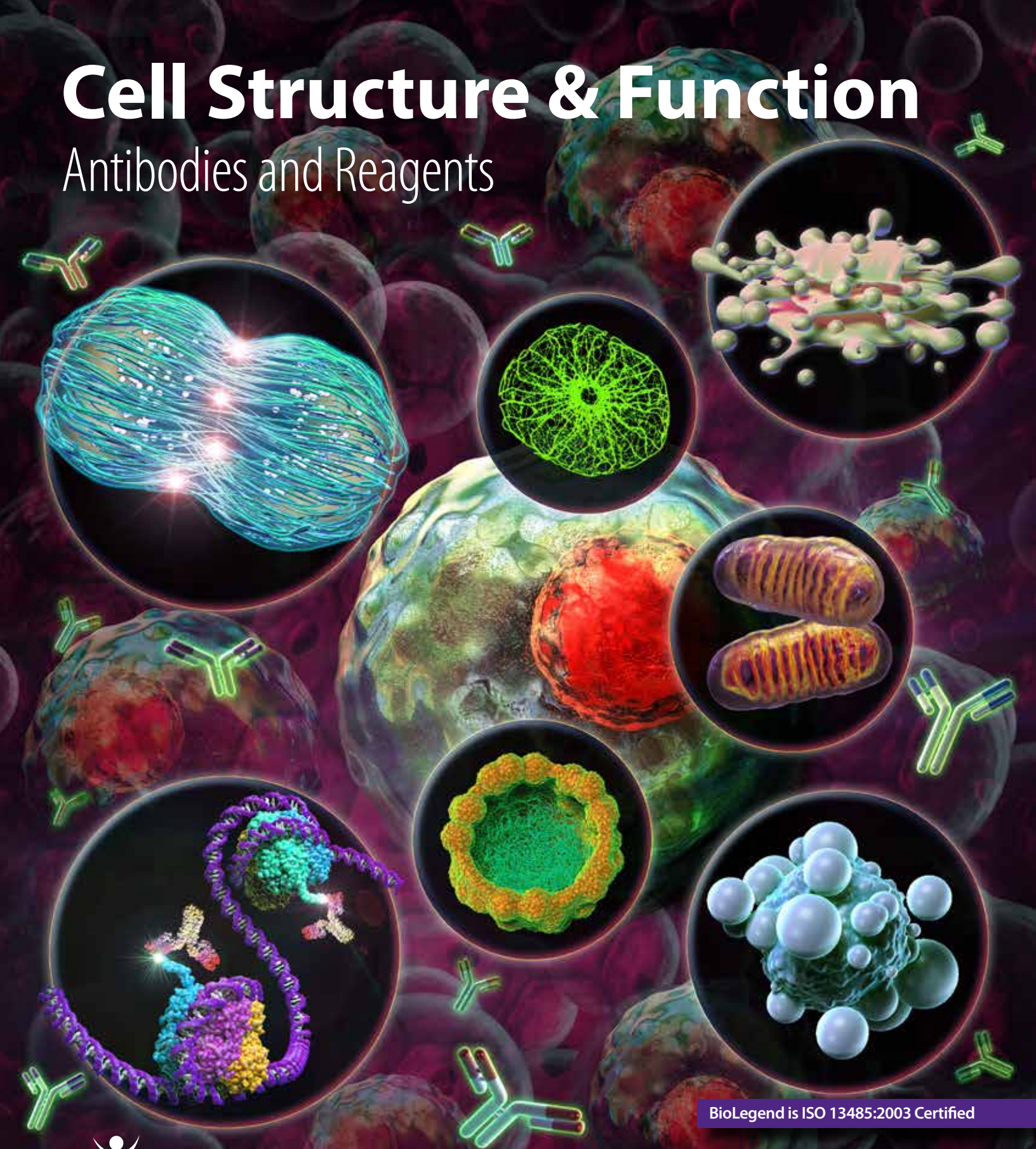
Cell Structure & Function

Antibodies and Reagents



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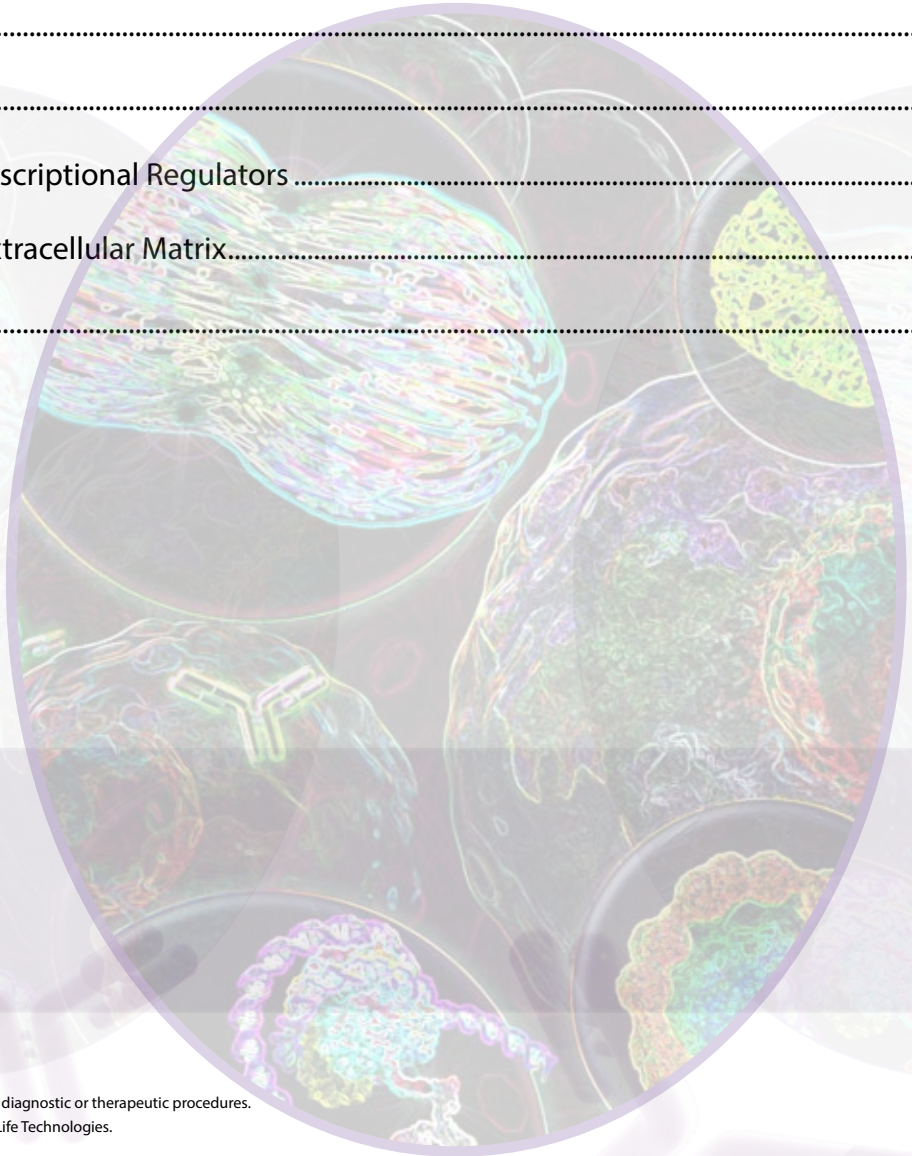


Introduction

BioLegend's growing portfolio of reagents for Cell Biology research now includes over 700 target proteins. Our reagents can be used to detect cellular structure proteins such as nuclei and golgi, and cell function proteins for processes such as cell proliferation, cell cycle, cell signaling, and epigenetics. These reagents can be used for many different types of assays such as western blot, microscopy, immunoprecipitation, ChIP, flow cytometry, and more, and are also applicable for several other research areas including Neuroscience, Immunology, Stem Cell research, and Cancer research.

BioLegend's reagents are supported by 100% product guarantee, superior customer service, and a quality management system that is certified by ISO 13485:2003. Our aggressive product development program is supported through internal hybridoma development, technology licensing, collaborations with the scientific community, and in-house product validation and testing.

To view all our Cell Biology product categories, visit:
biolegend.com/cell_biology

BioLegend Tools and Applications

Reagent	Primary and Secondary Purified and Conjugated Antibodies	Low Endotoxin, Azide-Free Antibodies for Bioassays	Recombinant Proteins	Immunoassay Solutions	Chemical Probes and Cellular Assay Kits	MojoSort™ Magnetic Cell Separation
Applications	For use in multiple applications including WB, IP, IHC, ICC, FC, ICFC, CyTOF®, ELISA, ChIP, or ELISPOT.	For use in functional assays such as cell activation, co-stimulation, blocking, or neutralization of cytokines.	For use in bioassays or as ELISA standards.	For quantification of single or multiple soluble analytes in standard ELISA using microplate reader or bead-based immunoassays suitable for flow cytometer.	Non-antibody based methods of detecting organelles or cell health based on chemical characteristics like hydrophobicity, charge, size and enzymatic activation.	For isolation and purification of cells from heterogeneous populations.
Web Link	biolegend.com/cell_biology biolegend.com/neuroscience biolegend.com/immunobiology	biolegend.com/LEAF	biolegend.com/recombinant_proteins	biolegend.com/bmia	biolegend.com/cell_health_proliferation	biolegend.com/mojosort

Cell Biology Antibody Validation

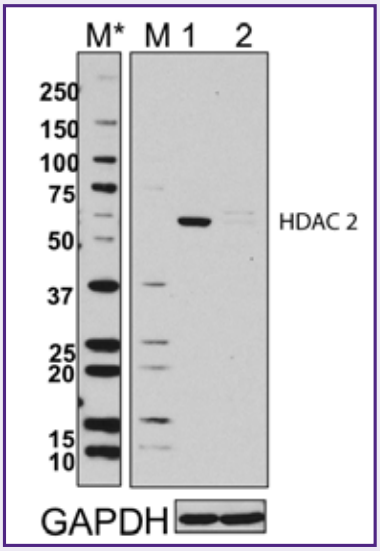
To ensure that we deliver the highest quality products, BioLegend's cell bio antibodies undergo extensive validation processes which include:

- Testing on knockout (KO) or knockdown (KD) cell lysates (CRISPR/Cas9 or siRNA), and negative control cell lines to confirm antibody specificity
- Testing on multiple species (human, mouse, rat, yeast, bacteria, zebrafish) whenever applicable, to determine antibody cross-reactivity
- Side-by-side comparison testing with competitor's antibody to ensure sensitivity
- Side-by-side comparison testing of lots with internal controls for lot-to-lot consistency
- Clones for one application (such as WB or ChIP) further validated in multiple applications (such as microscopy, flow cytometry) to provide the user the flexibility of supplementing their data with additional assays specific to their research
- Optimization of antibody suggested use by a titration range

Representative Data:

CRISPR/Cas9 Knockout

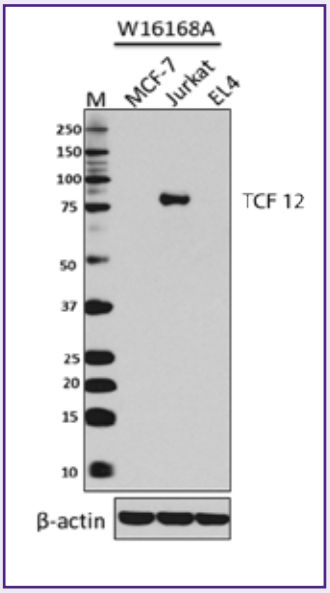
HDAC2



Total lysates (15 µg protein) from 293T (lane 1) and 293T/HDAC2 knockdown (KD) cells (lane 2) were resolved by electrophoresis (4-20% Tris-Glycine gel), transferred to nitrocellulose, and probed with 1:500 diluted (1 µg/mL) Purified anti-HDAC2 Antibody, clone 13G8C67 (upper) or 1:3000 diluted Purified anti-GAPDH Antibody, clone poly6314 (lower). Proteins were visualized by chemiluminescence detection using a 1:3000 diluted goat anti-mouse-IgG secondary antibody conjugated to HRP for the anti-HDAC2 Antibody, and a donkey anti-rabbit IgG Antibody conjugated to HRP for anti-GAPDH Antibody. Lane M: Molecular weight ladder, M* indicates longer exposure.

Negative Control Cell Line

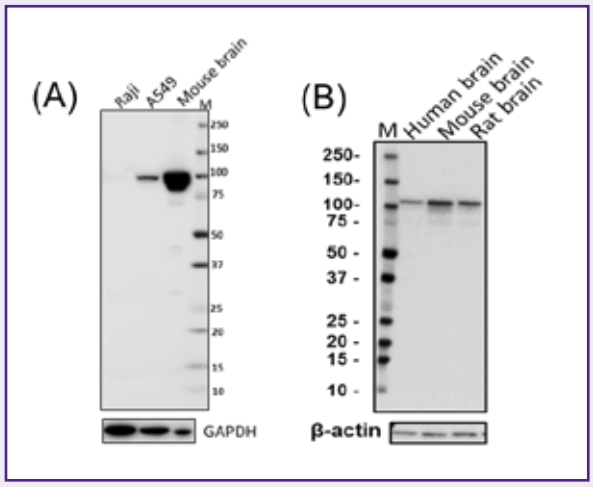
TCF12



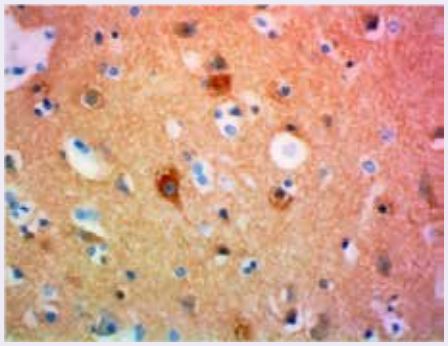
Total cell lysate (15 µg protein) from MCF-7 (negative control), Jurkat and EL4 cells were resolved by electrophoresis (4-12% Bis-Tris gel), transferred to nitrocellulose, and probed with 1:500 diluted (1 µg/mL) purified anti-TCF12 antibody (clone W16168A) (upper). Proteins were visualized by chemiluminescence detection using 1:3000 diluted HRP goat anti-rat-IgG secondary antibody for clone W16168A (upper). Direct-Blot™ HRP anti-β-actin antibody (1:2000 diluted, clone 2F1-1) was used as a loading control (lower). Lane M: Molecular weight ladder.

Validation of the same clone on multiple cell and tissue lysates, different applications, and different species for cross-reactivity.

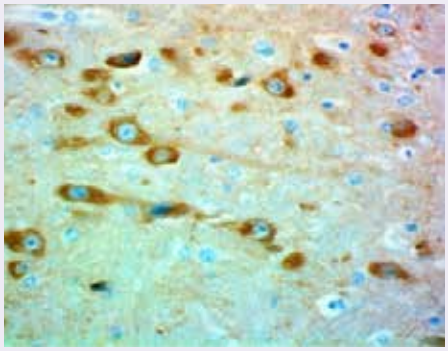
Dynamin-1



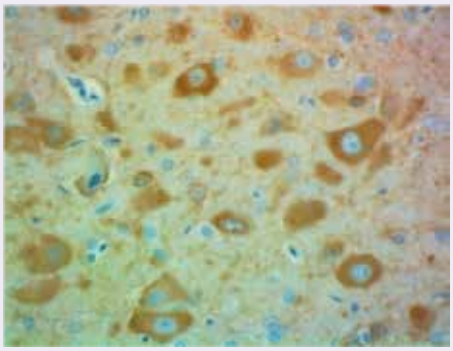
Total lysates (15 µg protein) from Raji (negative control), A549, and mouse brain, human brain and rat brain were resolved by electrophoresis, transferred to nitrocellulose, and probed with (A) 1:500 diluted (1 µg/mL) or (B) 1:250 diluted (2 µg/mL) purified anti-Dynamin-1 antibody (clone P83G4B6) (upper). Proteins were visualized by chemiluminescence detection using 1:3000 diluted HRP goat anti-mouse-IgG secondary antibody (Cat. No. 405306) for anti-Dynamin-1 antibody. 1:2000 (0.25 µg/mL) dilution of GAPDH (poly6314) antibody, or 1:1000 (0.5 µg/mL) dilution of β-actin (2F1-1) antibody was used as a loading control (lower). Lane M: MW ladder.



IHC staining of Formalin Fixed Paraffin Embedded (FFPE) normal human brain tissue. Following antigen retrieval using Sodium Citrate H.I.E.R (Cat. No. 928602), the tissue was incubated with anti-Dynamin-1 antibody (Clone P83G4B6) at 5 µg/mL overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB) (Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. Images were captured with a 40X objective.



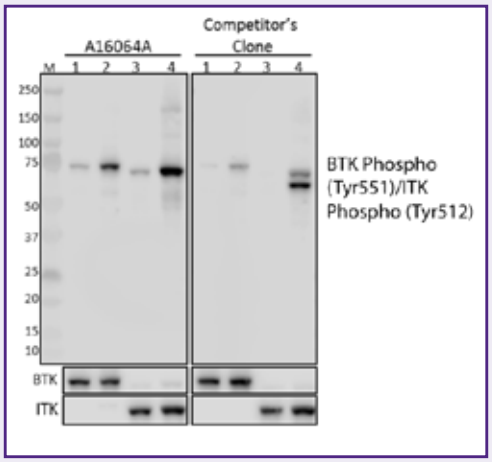
IHC staining of Formalin Fixed Paraffin Embedded (FFPE) mouse brain tissue. Following antigen retrieval using Sodium Citrate H.I.E.R (Cat. No. 928602), the tissue was incubated with anti-Dynamin-1 antibody (Clone P83G4B6) at 5 µg/mL overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB) (Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. Images were captured with a 40X objective.



IHC staining of Formalin Fixed Paraffin Embedded (FFPE) rat brain tissue. Following antigen retrieval using Sodium Citrate H.I.E.R (Cat. No. 928602), the tissue was incubated with anti-Dynamin-1 antibody (Clone P83G4B6) at 5 µg/mL overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB) (Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. Images were captured with a 40X objective.

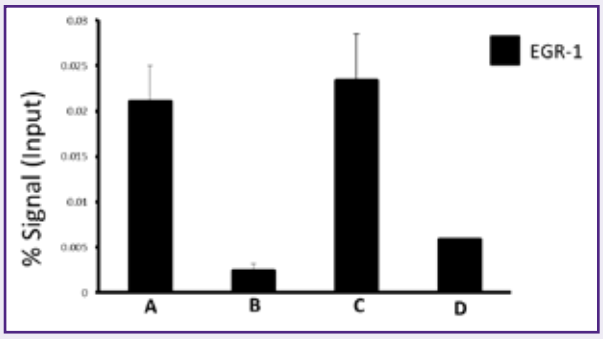
Compared to competitor's antibody side-by-side

BTK Phospho (Tyr551)/ITK Phospho (Tyr512)



Total cell lysates (15 µg protein) from Ramos cells treated without (lane 1) or with (lane 2) 2 mM H_2O_2 for 3 minutes, and Jurkat cells treated without (lane 3) or with (lane 4) the same stimulation were resolved by 4-20% Tris-Glycine gel electrophoresis, transferred to nitrocellulose, and probed with 1 µg/mL (1:500) purified anti-BTK Phospho (Tyr551)/ITK Phospho (Tyr512) antibody (Clone A16064A) or a competitor's clone used at the manufacturer's recommended concentration. Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse-IgG (Cat. No. 405301) for Clone A16064A and HRP donkey anti-rabbit IgG (Cat. No. 406401) for the competitor's clone. Membranes were then stripped and reprobed with an ITK antibody (Cat. No. 687302, 1 µg/mL, 1:500 dilution), and again stripped and reprobed with an antibody against BTK to confirm equal loading of the respective proteins.

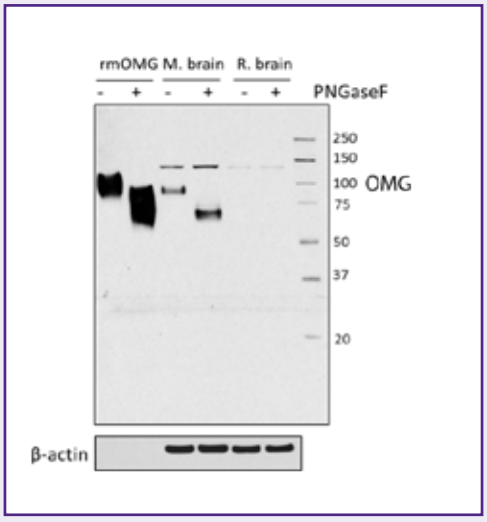
SSRP1



Chromatin Immunoprecipitation (ChIP) was performed using commercial Protein-G coated 96 well high-throughput ChIP assay kit by loading 3 µg of cross-linked chromatin samples from HeLa cells starved overnight and treated with 10% FCS with either A) 1:50 dilution of Go-ChIP-Grade™ Purified anti-SSRP1 antibody (Clone 10D1), B) equal amount of Purified Mouse IgG2b, κ Isotype Control antibody, or C) competitor's ChIP-grade Purified anti-SSRP1 antibody and D) equal amount of matched Isotype Control antibody as recommended by the manufacturer. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human EGR-1 gene region. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the 5% of total amount of input chromatin.

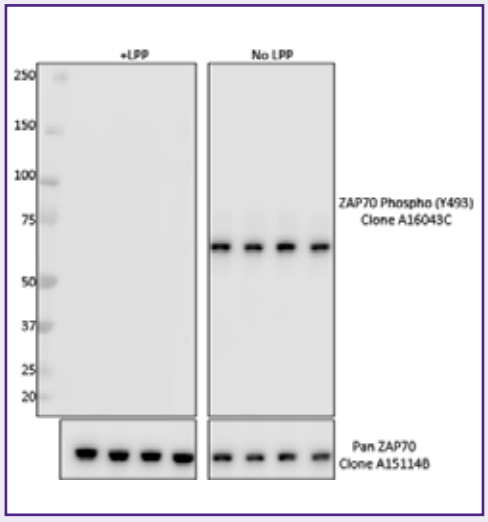
Cell treatment optimization

OMG



50 ng recombinant mouse OMG protein, mouse brain (M. brain) and rat brain (R. brain) total protein (15 µg each) were treated with or without 100 µg PNGase F at 37°C overnight to cleave the N-linked glycans from the proteins. All proteins were resolved by 4-12% Bis-tris gel electrophoresis, transferred to nitrocellulose, and probed with 1 µg/mL of purified anti-OMG antibody (clone A16003A). Proteins were visualized using a goat anti-rat-IgG secondary antibody conjugated to HRP and chemiluminescence detection. Direct-Blot™ HRP anti-β-actin antibody was used as a loading control.

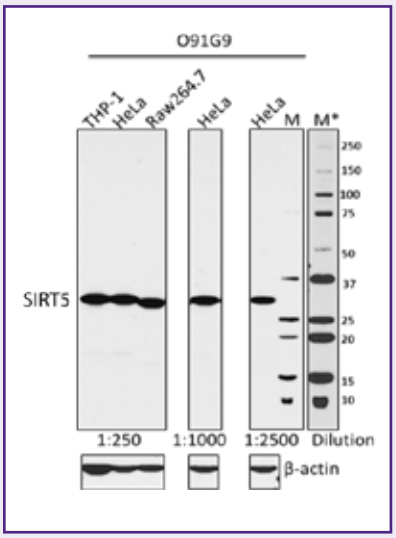
ZAP70 Phospho (Y493)



4 replicate whole cell extracts (15 µg total protein) prepared from Jurkat cells treated with 2 mM H_20_2 for 3 minutes were resolved by 4-20% Tris-Glycine gel electrophoresis and transferred to nitrocellulose membranes. Membranes were then treated with or without 30 units/mL of lambda protein phosphatase for 4 hours at room temperature. Each membrane was subsequently probed with 0.25 µg/ml (1:2000) purified anti-ZAP70 Phospho (Tyr493) antibody (Clone A16043C). Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse-IgG. ZAP70 loading was confirmed using purified anti-ZAP70 antibody used at 0.25 µg/ml (1:2000 dilution).

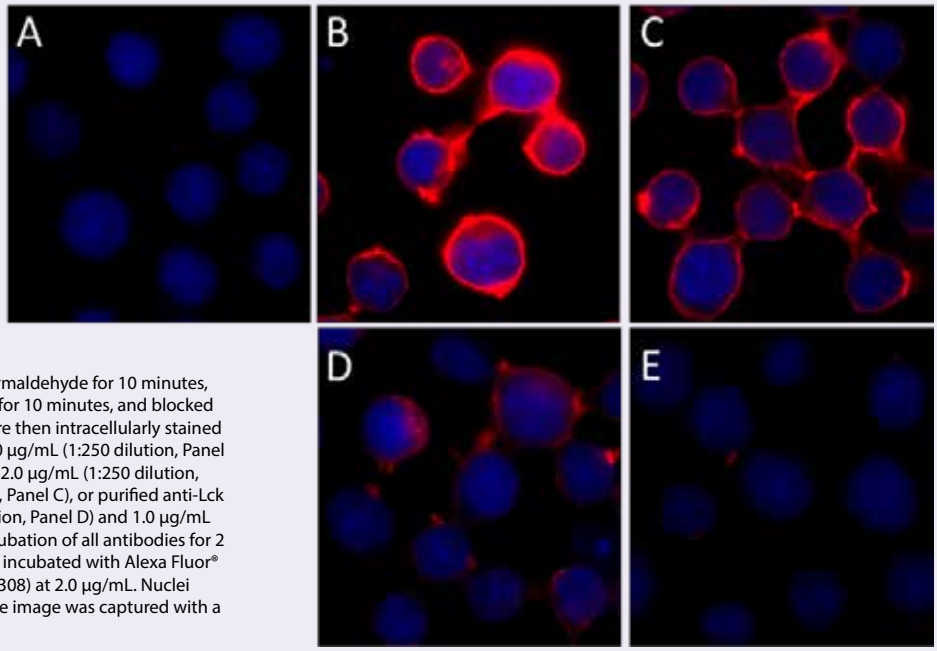
Antibody suggested use optimization by titration

SIRT5



Total cell lysate (15 µg protein) from THP-1, HeLa and Raw264.7 cells were resolved by electrophoresis (4-20% Tris-Glycine gel), transferred to nitrocellulose, and probed with 1:250 (2 µg/mL), 1:1000 (0.5 µg/mL) and 1:2500 (0.2 µg/mL) diluted purified anti-SIRT5 antibody (clone O91G9) or competitor's antibody used at manufacturer's recommended concentration (upper). Proteins were visualized by chemiluminescence detection using 1:3000 diluted HRP Goat anti-mouse IgG Antibody for clone O91G9 or 1:3000 diluted donkey anti-rabbit IgG Antibody conjugated to HRP for competitor's antibody (upper). 1:2000 dilution of Direct-Blot™ HRP anti-β-actin antibody (clone 2F1-1) was used as a loading control (lower). Lane M: Molecular weight ladder, M* indicates longer exposure.

Lck



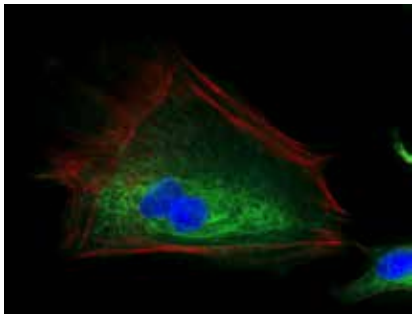
Jurkat cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 10% FBS for 60 minutes. Cells were then intracellularly stained with an isotype control antibody at 2.0 µg/mL (1:250 dilution, Panel A), purified anti-Lck clone A17013D at 2.0 µg/mL (1:250 dilution, Panel B) and 1.0 µg/mL (1:500 dilution, Panel C), or purified anti-Lck clone LCK-01 at 2.0 µg/mL (1:250 dilution, Panel D) and 1.0 µg/mL (1:500 dilution, Panel E). Following incubation of all antibodies for 2 hours at room temperature, cells were incubated with Alexa Fluor® 594 goat anti-mouse IgG (Cat. No. 405308) at 2.0 µg/mL. Nuclei were counterstained with DAPI and the image was captured with a 60X objective.

Cell Structure/ Organelles

M = Monoclonal Ab
P = Polyclonal Ab
FP = Functional Proteins
CP = Chemical Probes

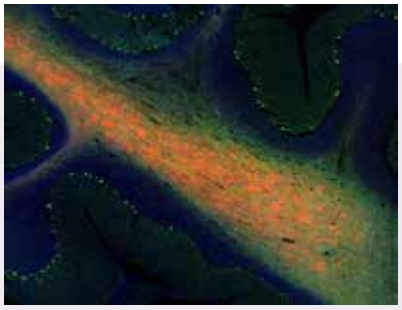
Cellular organelles are membrane bound structures within a cell that have unique cellular functions. BioLegend offers a number of products to detect sub-cellular localization of proteins through organelle-specific dyes, or specific antibodies, that can be used for live or fixed-cell imaging, as well as for western blot and flow cytometry.

Phalloidin is a very useful probe for imaging and stabilizing filamentous F-actin in fixed and permeabilized cells, providing structural and volumetric context to the cell.



Human paraffin-embedded cerebellum stained with Alexa Fluor® 647 anti-GFAP (red), Alexa Fluor® 488 anti-Tubulin Beta 3 (green), and DAPI (blue).

GFAP is an intermediate filament protein that is expressed by numerous cell types of the central nervous system (CNS) including astrocytes and ependymal cells and is involved in the structure and function of the cell's cytoskeleton.



C57BL/6 mouse frozen cerebellum section was stained with anti-GFAP (clone 2E1.E9) Alexa Fluor® 647 (red), followed with Flash Phalloidin™ Green 488 (green).

Cytoskeleton:

Specificity	M	P	FP	CP
Alpha-II Spectrin	•			
BRST-2	•			
Centrin 2 (Caltractin)	•			
Cytokeratin – 17	•			
Cytokeratin – 18	•	•		
Cytokeratin – 19	•			
Cytokeratin – 7	•			
Cytokeratin – 8	•			
Cytokeratin-pan	•			
Endoglin (CD105)	•		•	
Flash Phalloidin™ Green 488				•
Flash Phalloidin™ NIR 647				•
Flash Phalloidin™ Red 594				•
GFAP	•	•		
Keratin – 10	•	•		
Keratin – 6A		•		
Keratin 1		•		
Keratin 14		•		
Keratin 14		•		
Keratin 15		•		
Keratin 5		•		
Kinesin Heavy Chain	•			
MAP2	•	•		
Myosin Heavy Chain	•			
Myosin II – pan	•			
Nestin	•	•		
Nestin Tail	•			
Nestin, C-terminus	•			
Nestin, Repeat Region	•			
Neurofilament H & M (NF-H/ NF-M)	•			
Neurofilament H & M (NF-H/ NF-M), Phosphorylated	•			
Neurofilament H (NF-H)	•	•		
Neurofilament H (NF-H), Phosphorylated	•	•		
Neurofilament L	•			
Neurofilament M (NF-M)		•		
Neurofilament Marker (pan neuronal cocktail)	•			
NMHC II-C		•		
Non-muscle Myosin Heavy Chain II-B		•		
Vimentin	•	•		
α-Skeletal Muscle Actin	•			
α-Smooth Muscle Actin	•			
α-Tubulin	•			
β-Actin	•	•		
β-Tubulin	•			
β-Tubulin IIb (TUBB2)	•			
β-Tubulin III (TUBB3)	•	•	•	
γ-Tubulin		•		

Nuclear Markers:

Specificity	M	P	FP	CP
7-AAD				•
Centrin 2 (Caltractin)	•			
CytoPhase™ Violet				•
DAPI				•
DRAQ5™				•
DRAQ7™				•
Fibrillarin	•			
Helix NP™ Blue				•
Helix NP™ Green				•
Helix NP™ NIR				•
Lamin A		•		
mAKAP	•			
Nuclear Pore Complex Proteins/ NUP98	•			
Nucleolin-Phospho (Thr76/ Thr84)	•			
NUP153	•			
PCNA	•			
Propidium Iodide				•

Lysosome Markers:

Specificity	M	P	FP	CP
CD63/LAMP-3	•			
CD107a (LAMP-1)	•			
CD107b (Mac-3)/LAMP-2	•			
CD63/LAMP-3	•			
Cathepsin A	•		•	
Cathepsin B	•		•	
Cathepsin D	•		•	
Cathepsin E (CTSE)			•	
LAMP 5	•			

Mitochondrial Markers:

Specificity	M	P	FP	CP
BAP37		•		
Cytochrome c	•			
DJ-1 (PARK7)	•	•		
Grp75 (Mortalin)	•			
HSD17B10	•			
HSP60	•			
LRRK2	•			
LRRK2 (NH2-terminus)	•			
Mitofusin-1	•			
Mitofusin-2	•			
MitoSpy™ Green FM				•
MitoSpy™ Orange CMTMRos				•
MitoSpy™ Red CMXRos				•
Nhedc2 (NHA2)		•		
Pancortin	•			
PARIS (ZNF746)	•			
PINK1	•			
Prohibitin		•		
TFAM	•			
UQCRC1	•			
VDAC1	•			

Ribosome:

Specificity	M	P	FP	CP
RPS6	•			
p90 Rsk		•		

Golgi:

Specificity	M	P	FP	CP
Giantin		•		
GPP130		•		

Endoplasmic Reticulum:

Specificity	M	P	FP	CP
GRP94		•		
PERK Phospho (Ser713)		•		

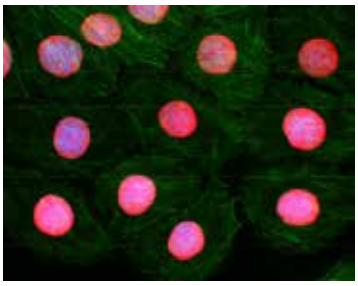
Centrosome Markers:

Specificity	M	P	FP	CP
Aurora A (Aurora 2)		•		
Aurora A (Aurora 2)-Phosphorylated (Thr288)		•		
Aurora B	•			
Ninein		•		
Pericentrin		•		

Autophagosomes:

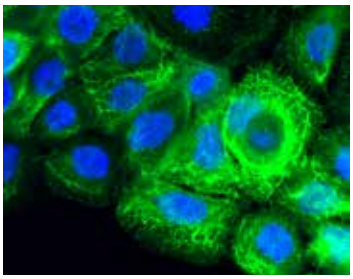
Specificity	M	P	FP	CP
ATG5	•			
ATG17	•			
HSC70	•			
HSF1	•			
HSF2	•			
Hsp70	•			
Hsp90α	•			
Hsp90α/β	•			
Hsp90α/β	•			
LAMP1 (CD107a)	•			
LAMP2 (CD107B)	•			
LAMP2 (CD107B)	•			
LC3	•			
p62 (SQSTM1)	•			
p62	•	•		
Parkin	•			
PINK1	•			
Ubiquitin	•			

Lamin A is a member of the intermediate filament family, and is thought to function as a fibrous component of the nuclear lamina, providing a framework for the nuclear envelope, and possibly interacting with chromatin.



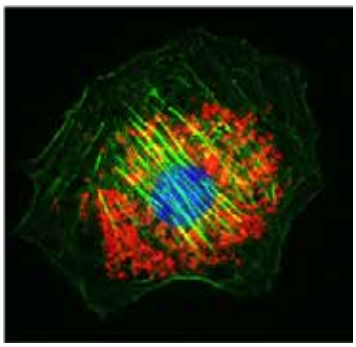
HeLa cells were stained with anti-Lamin A Antibody (clone poly6135) followed by Alexa Fluor® 594 (red) conjugated goat anti-Rabbit IgG. Actin filaments were labeled with Alexa Fluor® 488 Phalloidin (green). Nuclei were counterstained with DAPI (blue).

Keratin 5 is a member of the type II (basic or neutral) cytokeratin family that are heteropolymeric structural proteins coexpressed during differentiation of simple and stratified epithelial tissues.

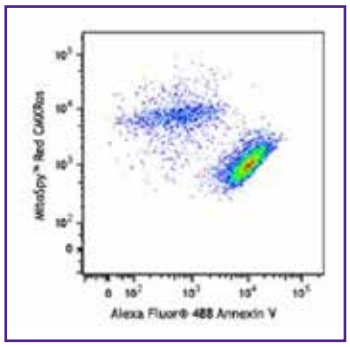


A431 cells were stained with anti-Keratin 5 antibody (clone Poly19055) and Alexa Fluor® 488 (Green) secondary antibody. Nuclei were counterstained with DAPI (Blue).

MitoSpy™ mitochondrial localization probes are cell-permeant, fluorogenic chemical reagents that are used for labeling mitochondria in living cells.

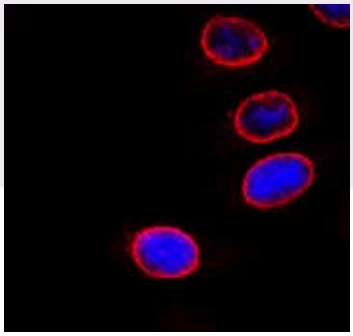


NIH3T3 cells were stained with MitoSpy™ Red CMXRos (red), fixed and permeabilized with 1X True Nuclear™ Perm Buffer. Then the cells were stained with Flash Phalloidin™ NIR 647 (green) and counterstained with DAPI (blue).



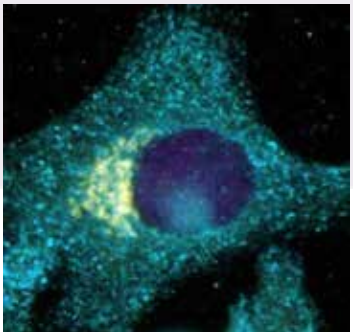
Jurkat cells were treated with LEAF™ purified anti-human CD95, and stained with MitoSpy™ Red CMXRos following by staining with Alexa Fluor® 488 Annexin V.

Nuclear pores are large protein complexes that cross the nuclear envelope and allow the transport of molecules across the nuclear envelope.



HeLa cells were stained with 1 µg/ml Nuclear Pore Complex Proteins antibody (clone MAb414). Alexa Fluor® 594 (Red) Goat anti-Mouse IgG was used as secondary antibody. Nuclei were counterstained with DAPI (Blue).

Clathrin is the most abundant protein in clathrin-coated vesicles that are involved in multiple vesicle trafficking pathways, and facilitate intracellular transport of cargo proteins following endocytosis.

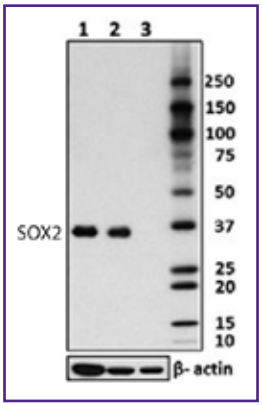


MeOH fixed HeLa cells double stained with Clathrin (clone TD.1), and Giantin (Poly19243). Cells were counterstained with DAPI.

Cell Development and Differentiation

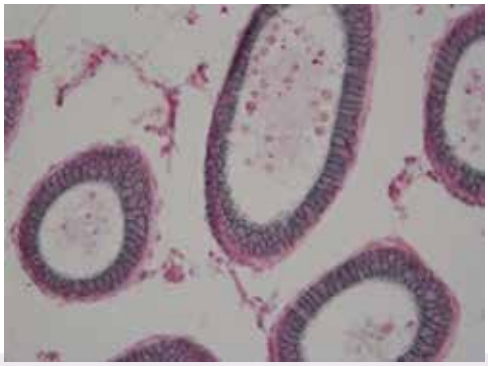
In developmental biology, cellular differentiation is the process where a cell changes from one cell type to another. These changes are largely due to highly controlled modifications in gene expression, and signaling cascades in the cell which include TGF-β/BMP signaling, Wnt/β-catenin signaling, Notch pathway, Hedgehog signaling, and Hippo signaling pathways. Cell differentiation not only occurs when a zygote transforms into a complex organism, but also continues into adulthood when adult stem cells fully differentiate into daughter cells during tissue repair and normal cell turnover. Because stem cells can replicate as well as differentiate to give rise to a variety of cell types, they are of considerable interest for potential medical applications.

SOX2 is involved in the regulation of embryonic development and in the determination of cell fate.



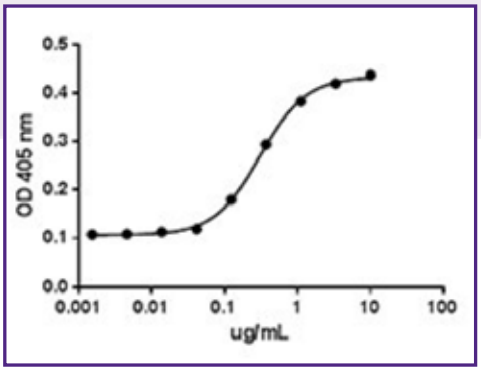
Cell lysates from NTERA-2 cells (lane 1), NF-1 (lane 2), and HepG2 cells (lane 3) were probed with purified anti-SOX2 antibody (poly6519). Direct-Blot™ HRP anti-β-actin antibody (clone 2F1-1) was used as a loading control.

β-catenin plays a key role in Wnt signaling pathways and thus is involved in neural differentiation, synaptic plasticity, and neurodegenerative disease.



Paraffin-embedded mouse testicle sections stained with anti-β Catenin 1 antibody (clone 12F7).
Credit: Hans Snyder, Histologics.

Human Sonic Hedgehog (SHH) is expressed in embryonic tissues and is critical for the patterning of early embryos.



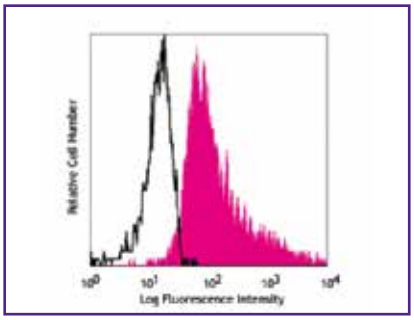
Recombinant Human SHH protein induces C3H10T1/2 differentiation in the presence of hBMP9 as measured by alkaline phosphatase production.

M = Monoclonal Ab
P = Polyclonal Ab
FP = Functional Proteins
E = ELISA
LP = LEGENDplex™

Specificity	M	P	FP	E	LP
β Catenin 1 (CTNNB1)	•				
Bax	•				
B7-H1/PD-L1	•		•		
Bcl-11b					
BDNF			•		
BMP-4,5,6,7,9,10,13,14	•		•		
CD30 (TNFRSF8)-Fc Chimera	•		•		
CD30L	•		•		
CD117 (c-kit)	•			•	
CD133	•				
CD135 (Flt-3, Flk-2)	•		•		
CD349 (Frizzled-9)	•				
CD90/Thy1	•				
c-MAF	•				
DLL1	•		•		
DLL4	•				
DHH			•		
DKK-1			•		
Endoglin	•		•		
FGF-3,4, 19	•		•		
FGF-6, 9,10, 17, 18, 21			•		
FOX3 (NeuN)					
FOXA2	•				
FoxP3		•			
GATA3	•				
GFAP	•	•			
Gli-1		•			
Helios	•				
HMGB1	•		•		
IGFBP-1,6,7			•		•
IGFBP-3	•		•		•
IGFBP-4	•		•		•
IGFBP-5			•		
Ikaros	•				
Jagged 1,2	•				
Lin-28A	•				
Nanog	•				
Nestin	•	•			
NKX2-1 (TTF-1)	•				
Notch-1	•				
Notch-2	•				
Notch-3	•				
Notch-4	•				

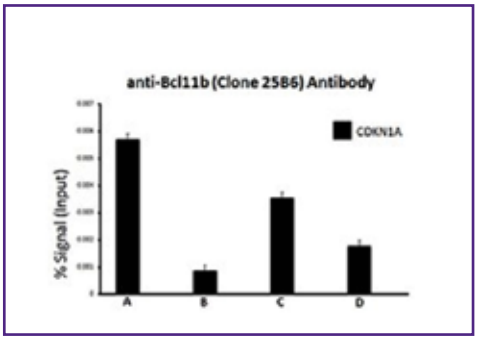
Specificity	M	P	FP	E	LP
Oct4	•				
p53	•				
p63 (TA)		•			
p63 (ΔN)		•			
Pax-2, 5, 6, 9	•				
PCNA	•				
PLZF	•				
Prox1	•				
PTEN	•				
RUNX1	•				
Sca-1/Ly6A/E	•				
SHIP-1	•				
SCF	•	•	•	•	•
Smad6			•		
Sonic Hedgehog	•				
SOX2	•	•			
SPI1 (PU.1)	•				
SSEA-1, 3, 4, 5	•				
STAT1	•				
STAT1 Phospho (Ser727)	•				
STAT3	•	•			
STAT3 Phospho (Tyr705)	•				
STAT5	•				
STAT5 Phospho (Tyr694)		•			
STAT6	•				
STAT6 Phospho (Tyr641)	•				
Syk	•				
T-bet	•				
TCF3 (E2A)	•				
TGF-α			•		•
TGF-β	•		•	•	•
TMTSP (THSD1)	•				
TNAP	•				
TRA-1-60-R/ Podocalyxin	•				
TRA-1-81	•				
TRA-2-49	•				
TRA-2-54	•				
TSG			•		

Notch 4 is expressed by a variety of tissues including heart, lung, placenta, and liver, and controls many developmental processes. It functions as a receptor for transmembrane ligands such as the Jagged and Delta proteins.



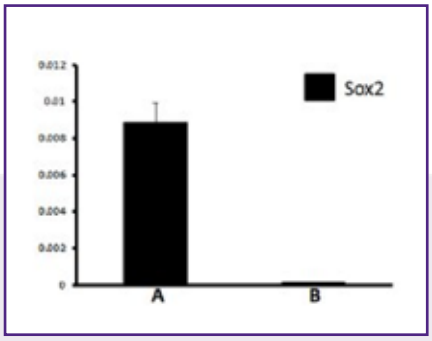
Human acute lymphoblastic leukemia cells HPB-ALL stained with PE anti- human Notch 4 (clone MHN4-2 PE).

Bcl-11b is a C2H2-type zinc finger protein that is expressed in thymus, mainly T cells. It is indispensable for T lineage development.



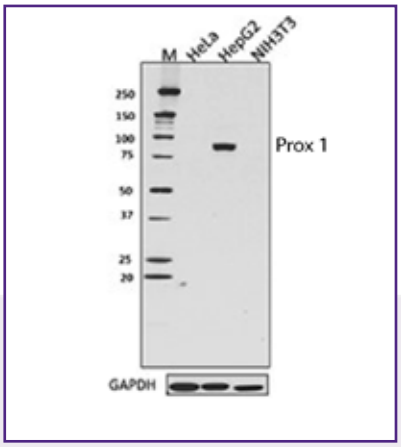
Chromatin Immunoprecipitation (ChIP) was performed using Jurkat cells with either A) Go-ChIP-Grade™ purified anti-Bcl11b antibody, B) equal amount of isotype control antibody, or C) competitor's ChIP-grade purified antibody, and D) equal amount of matched isotype control antibody as recommended by the manufacturer. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human CDKN1A gene regions, which is known to be bound to Bcl11b.

Oct4 (Octamer binding transcription factor 4) is an important marker of the undifferentiated state and central regulator of pluripotency in ES cells. When embryonic stem cells are triggered to differentiate, Oct4 is downregulated, thus providing a model for the early events linked to somatic differentiation in the developing embryo.



Chromatin Immunoprecipitation (ChIP) was performed using NCCIT cells with either A) Go-ChIP-Grade™ Purified anti-Oct4 (Oct3) (clone 3A2A20), or B) equal amount isotype control antibody. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human Sox2 gene regions.

Prox1 is a homeobox transcription factor responsible for progenitor cell differentiation and the development of several organs, such as those in the central nervous system and the liver.



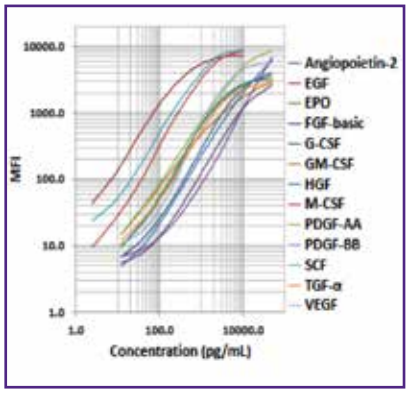
Cell lysates from HeLa (negative control), HepG2 and NIH3T3 cells were probed with purified anti-Prox1 (clone W16098A) antibody, or loading control anti-GAPDH (poly6314) antibody. Lane M: molecular weight ladder.

Growth Factors and Receptors

M = Monoclonal Ab
P = Polyclonal Ab
FP = Functional Proteins
E = ELISA
LP = LEGENDplex™

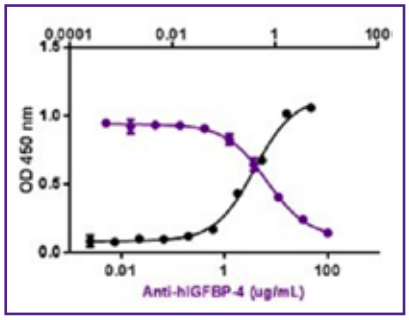
The progression of cells through cell division, cell growth, proliferation, and differentiation is regulated both by extracellular signals from the environment, as well as by internal signals that monitor and coordinate the various processes. An example of cellular regulation by extracellular cues is provided by growth factors that act through growth factor receptors on the cell surface. The effects of growth factors on the cell are carried down by several signaling pathways including the JAK/STAT, MAP-Kinase, and the PI3-Kinase pathways.

The LEGENDplex™ Human Growth Factor Panel is a bead-based multiplex assay, using fluorescence-encoded beads suitable for use on various flow cytometers.



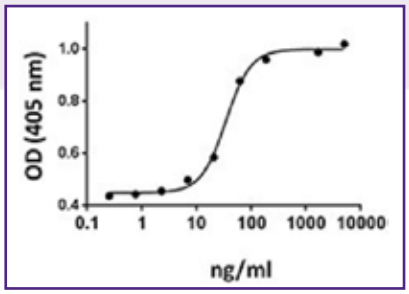
Standard curve generated using the LEGENDplex™ Human Growth Factor Panel.

IGFBP-4 binds both insulin-like growth factors (IGFs) I and II, and prolongs the half-life of the IGFs, as well as alters their interaction with cell surface receptors.



Recombinant human IGFBP-4 binds human IGF-I in a dose-dependent manner (black circles). The binding of human IGF-I to human IGFBP-4 is blocked (purple circles) by increasing concentrations of Ultra-LEAF™ Purified anti-human IGFBP-4 antibody (Clone A15038E).

Bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily and play a key role in embryonic development.

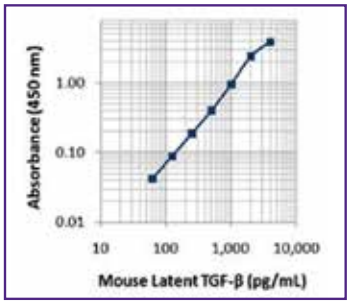


Recombinant human BMP-6 protein induces alkaline phosphatase production in the mouse chondrogenic ATDC5 cells.

Specificity	M	P	FP	E	LP
Activin A	•		•		
Amphiregulin	•		•		
Angiopoietin-2	•		•		•
Artemin			•		
Asprosin			•		
BAFF	•		•		•
BAFFR	•		•		
BDNF			•		
Betacellulin	•		•		
Betatrophin	•				
BMP-4			•		
BMP-5			•		
BMP-6			•		
BMP-7			•		
BMP-9			•		
BMP-10			•		
BMP-13			•		
BMP-14 (GDF-5)			•		
CD27L	•		•		
CD40L (TNFSF5)	•		•		•
CNTF			•		
CRP	•		•		•
DHH			•		
EGF	•		•	•	•
EG-VEGF			•		
Epigen			•		
Epiregulin			•		
EPO	•		•	•	•
FGF-1-acidic	•		•		
FGF-3	•		•		
FGF-4	•		•		
FGF-6			•		
FGF-9			•		
FGF-10			•		
FGF-17			•		
FGF-18			•		
FGF-19	•				
FGF-21			•		
FGF-basic	•		•	•	•
FLT3L			•		
G-CSF	•		•		•
GDNF			•		
GM-CSF	•		•	•	•
HB-EGF			•		
HGF	•	•	•		•

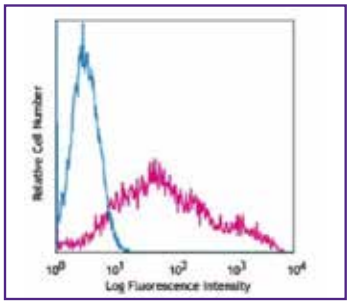
Specificity	M	P	FP	E	LP
IGF-I	•		•		
IGF-II	•		•		
IGFBP-4	•		•		•
Leptin	•			•	
LIF	•	•	•	•	
Light (TNFSF14)	•		•		
M-CSF	•		•		•
Midkine			•		
Neurturin			•		
NGF	•		•		
NGFR	•				
NNT-1 (BCSF-3)			•		
Noggin			•		
NRG1 (Heregulin) EGF Domain (CF)			•		
NT-3			•		
NT-4			•		
Oncostatin M	•		•		
OX40L	•		•		
PDGF-AA			•		•
PDGF-BB			•		•
PDGF-CC			•		
PDGFRa, b	•		•		
Persephin			•		
PLGF-1			•		
PTH	•		•		
Prolactin	•		•		
RANK (TNFRSF11A)	•		•		
RBP4	•		•		•
S100A8/A9 Heterodimer	•		•	•	•
SCF	•	•	•	•	•
Slit2-N			•		
Sonic Hedgehog			•		
TGF-α			•		•
TGF-β	•		•	•	•
Thrombopoietin (TPO)	•		•		
TNFSF18 (GITRL)	•		•	•	
TRANCE (RANKL)	•		•		
TSLP	•	•	•	•	•
VEGF	•	•	•		•
WISP-1			•		
WNT-7a			•		

TGF-β is a cytokine that has critical functions in the immune response by regulating Treg and Th17 cells.



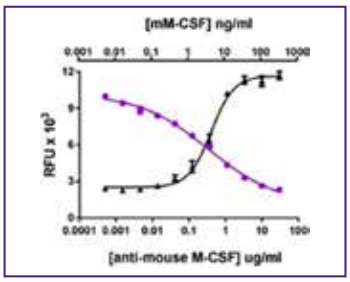
ELISA assay using biotin anti-mouse LAP (TGF-β1) antibody (clone TW7-16B4) as the detection antibody to generate the example standard curve.

BAFF, also known as B cell activating factor, tumor necrosis factor ligand superfamily 13B, B lymphocyte stimulator (BLYS), and TNF homolog, activates apoptosis, NF-κB, and JNK (THANK). BAFF is a type II transmembrane protein, and a member of the tumor necrosis factor ligand superfamily.



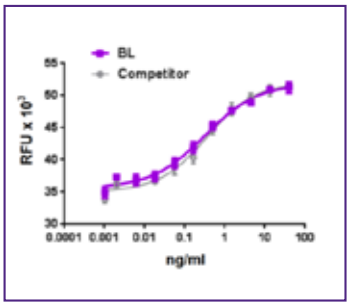
IFN-γ stimulated THP-1 (human monocytic cell line) stained with PE anti-human CD257 (BAFF, BLYS) Antibody (clone T7-241).

M-CSF is a secreted glycoprotein that induces monocyte and macrophage colony formation from precursors in murine bone marrow cultures.



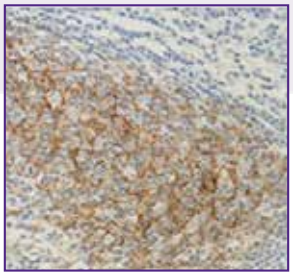
Recombinant mouse M-CSF induces the proliferation of mouse myelogenous leukemia lymphoblast M-NFS-60 cells in a dose dependent manner (black triangles). Ultra-LEAF™ Purified anti-mouse M-CSF antibody (clone A16063L, purple circles) neutralizes the proliferation of M-NFS-60 cells induced by recombinant mouse M-CSF.

Human oncostatin M (OSM) was initially isolated from supernatant of U937 cells treated with PMA. It was identified by its property to inhibit the proliferation of A375 melanoma cells and other human tumor cells.



Recombinant human oncostatin M induced the proliferation of human erythroleukemic TF-1 cells in a dose dependent manner. BioLegend's protein was compared side-by-side a competitor's equivalent product.

NGFR is expressed by many cell types including neurons and Schwann cells that promote cell apoptosis and regulate cell differentiation and neurogenesis.



Staining of Clone NGFR5 on formalin-fixed paraffin-embedded normal human tonsil tissue.

Cell Proliferation, Growth, and Viability

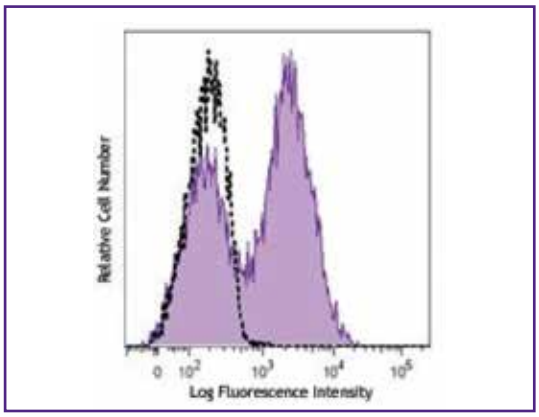
Cell growth and proliferation are among the most fundamental biological processes. The core cell cycle machinery that drives these processes have been conserved from yeast to humans. Proper spatial and temporal growth and proliferation is essential to allow normal development of organisms, and to maintain physiological homeostasis. Abnormal and uncontrolled cell proliferation leads to pathological conditions such as cancer.

Cell proliferation and viability can also be used to assess cell health. Different types of assays are used to detect these processes, such as using antibodies specific to proliferation and growth markers, measuring DNA synthesis, detecting metabolic activity, and/or cellular ATP/GTP levels.

Specificity	M	P	CP
ATPase Assay Kit			•
BrdU	•		•
Calcein Violet-AM			•
Calcein-AM			•
CFSE Cell Division Tracker Kit			•
CytoPhase™ Violet			•
Deep Blue Cell Viability™ Kit			•
GTPase Assay Kit			•
Helix NP™ Blue			•
Helix NP™ Green			•
Helix NP™ NIR			•
Ki-67	•		
LDH-Cytox™ Assay Kit			•
p53	•		
PCNA	•		
Tag-it Violet™ Proliferation and Cell Tracking Dye			•
TetraZ™ Cell Counting Kit			•

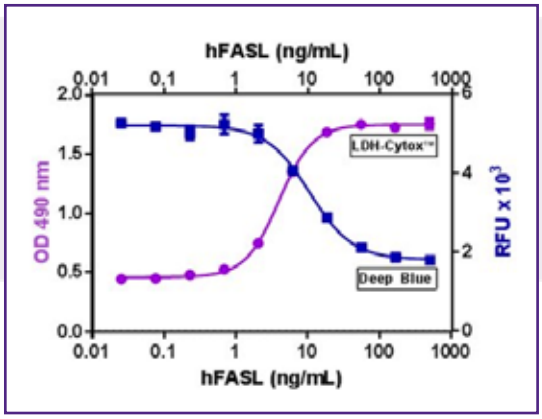
M = Monoclonal Ab
P = Polyclonal Ab
CP = Chemical Probes

Nuclear protein Ki-67 plays an essential role in ribosomal RNA transcription and cell proliferation, and is strongly expressed in proliferating cells and has been reported as a prognostic marker in various tumors.



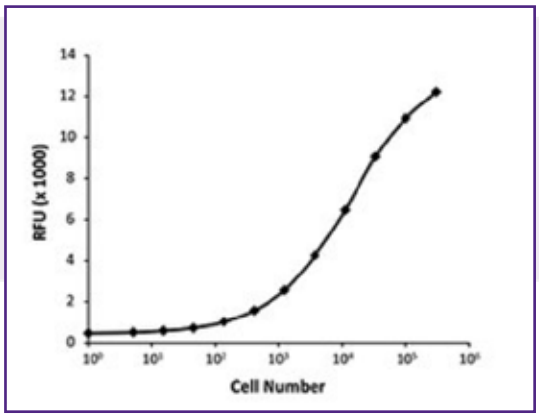
Con A+IL-2-stimulated C57BL/6 mouse splenocytes stained with Ki-67 (clone 16A8) Brilliant Violet 421™ (filled histogram) or rat IgG2a, κ Brilliant Violet 421™ isotype control (open histogram).

LDH-Cytox Assay™ Kit is a kit for determination of cytotoxicity by measuring lactate dehydrogenase activity released from damaged cells.



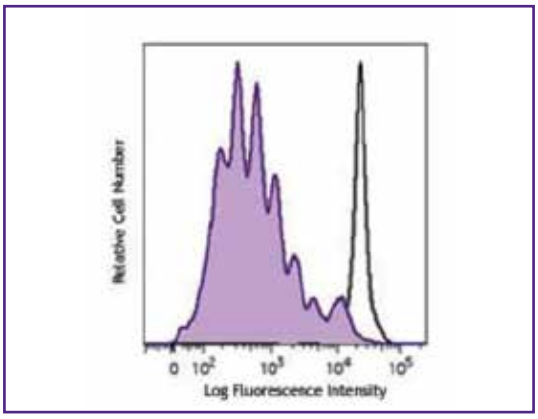
Human FASL induced cytotoxicity in Jurkat cells is measured using both LDH-Cytox™ Assay Kit (OD 490nm) and Deep Blue Cell Viability™ Kit (RFU x 103).

The Deep Blue Cell Viability™ Kit is formulated to study cell proliferation and quantification through the extent of resazurin reduction and resorufin production which is proportional to the number of metabolically active cells (live cells) present in the culture.



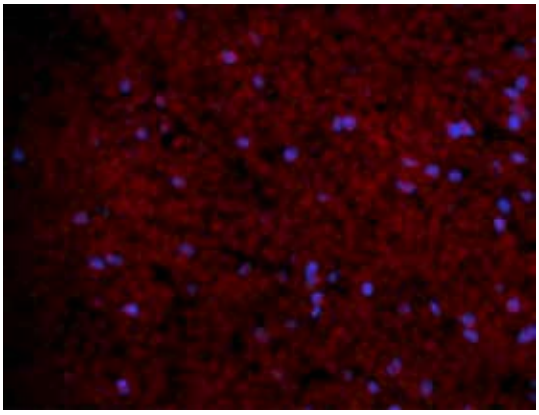
Detection of Baf3/CCR3 cells viability using Resazurin fluorescence measurement. The increasing cell numbers correlate with the increasing fluorescence.

CFSE (formally known as 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester of CFDA SE) is widely used for cell proliferation assays and *in vivo* cell tracking.



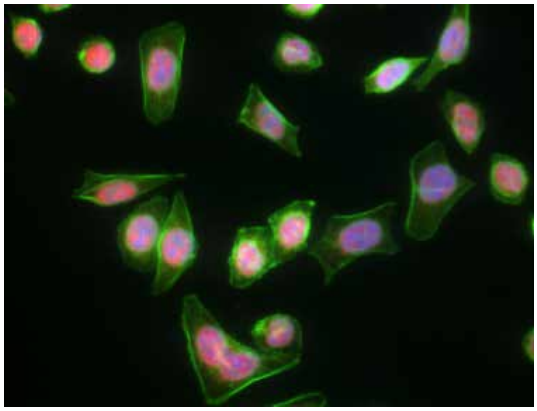
Human peripheral blood mononuclear cells were stained with CFSE Cell Division Tracking Kit, and then stimulated with (filled histogram) or without (open histogram) PHA for 5 days. On day 5, cells were harvested and the CFSE fluorescent staining was analyzed by flow cytometry.

Tag-it Violet™ Proliferation and Cell Tracking Dye can be used for cell tracking and for proliferation assays.

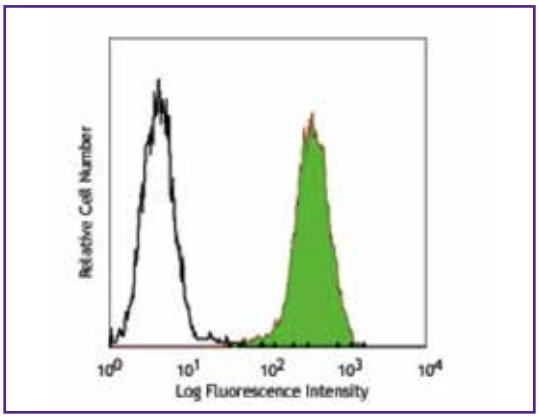


Mouse spleen 72 hours after adoptive transfer of Tag-it Violet™-labeled splenocytes (purple). Nucleated cells are stained using 25 µM DRAQ™ (red).

Proliferating cell nuclear antigen also known as PCNA or the DNA polymerase δ auxiliary protein, is a 36 kD trimeric ring that acts as a DNA-polymerase sliding clamp expressed in the nucleus of all proliferating cells.

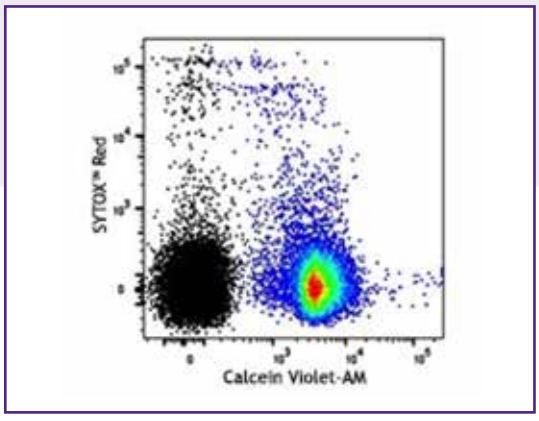


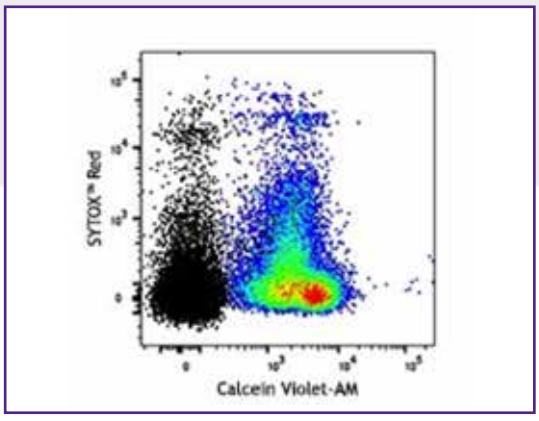
HeLa cells were fixed with 1% paraformaldehyde (PFA) for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 5% FBS for 30 minutes. Then the cells were intracellularly stained with 1 µg/ml anti-human/mouse/rat PCNA (clone PC10) Alexa Fluor® 594 (red) in blocking buffer, overnight at 4°C, followed by Alexa Fluor® 488 Phalloidin (green) staining for 20 minutes. Nuclei were counterstained with DAPI (blue).



MOLT-4 cells fixed in 70% ethanol then stained with PE anti-human/mouse/rat PCNA Antibody (clone PC10).

Calcein Violet-AM is a fluorogenic, cell-permeant fluorescent probe that indicates cellular health by detecting the activity of nonspecific esterases.



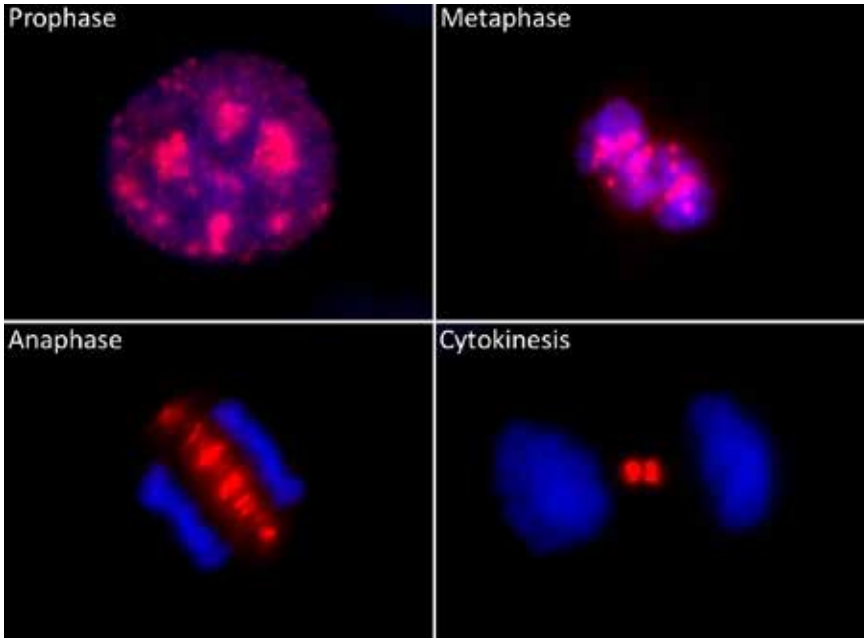


Fresh (left) or day-old C57BL/6 splenocytes (right) were stained with Calcein Violet-AM and a cell-impermeant nucleic acid dye, SYTOX™ Red (colored). Black figure represents unstained splenocytes.

Cell Cycle

The cell cycle is a series of precisely timed and carefully regulated stages in a cell that consists of cell growth, DNA replication, distribution of the chromosomes to daughter cells, and cell division. It consists of four distinct phases: Go (resting) /G1 (post-mitotic gap) phase, S (synthesis) phase, G2 (growth) phase, and M (mitosis) phase. A large number of proteins are involved in the complex machinery that regulate and maintain the cell cycle such as cyclins, cyclin-dependent kinases, tumor suppressors, and checkpoint proteins. These proteins ensure that the cell cycle continues only after the completion of a prior stage. In the event of a DNA damage, checkpoint proteins are activated and checkpoint-arrested cells resume cell-cycle progression once the DNA damage has been repaired. Cells with irreparable DNA lesions undergo permanent cell-cycle arrest or apoptosis.

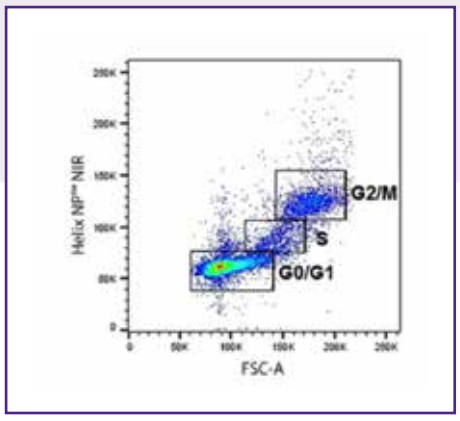
Aurora B plays a critical role in cell division and chromosome-microtubule interactions during mitosis. The expression level of Aurora B is dramatically increased during the S/G2/M phases of the cell cycle.



HeLa cells were intracellularly stained with anti-Aurora B antibody (clone W16153A) followed by Alexa Fluor® 594 (red) conjugated goat anti-rat IgG (Cat. No. 405422). Nuclei were counterstained with DAPI (blue). The images displayed HeLa cells at different phases of mitosis.

M = Monoclonal Ab
P = Polyclonal Ab
CP = Chemical Probes

Specificity	M	P	CP
Alpha B Crystallin		•	
APC7		•	
Artemis	•		
Aurora A (Aurora 2)		•	
Aurora A (Aurora 2)-Phosphorylated (Thr288)		•	
Aurora B	•		
BrdU	•		•
C2H2 zinc finger protein phospho linker region (HpTGEKP)	•		
C/EBPα	•		
Centrin 2 (Caltractin)	•		
Cdc2 (p34)	•		
Cdk2	•		
Cdk4		•	
CDK5			
CDK7 Phospho (Ser164/Thr170)	•		
CDKN1A	•		
CDKN2A	•		
Centrin 2	•		
CFSE Cell Division Tracker Kit			•
Chk2	•		
ch-TOH		•	
c-Myc	•		
Cyclin A	•		
Cyclin B1	•		
Cyclin D1	•		
Cyclin D2			
Cyclin D3	•		
CytoPhase™ Violet			•
DAPI			•
DNA-PKcs Phospho (Thr2609)	•		
DRAQ5™			•
DRAQ7™			•
Eg5	•		

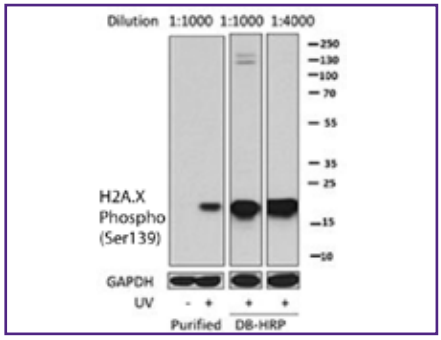


Helix NP™ NIR is a far-red emitting nucleic acid stain that can be used to stain fixed cells for cell cycle analysis.

C57BL/6 mouse thymus cells were fixed using chilled ethanol and stained with Helix NP™ NIR.

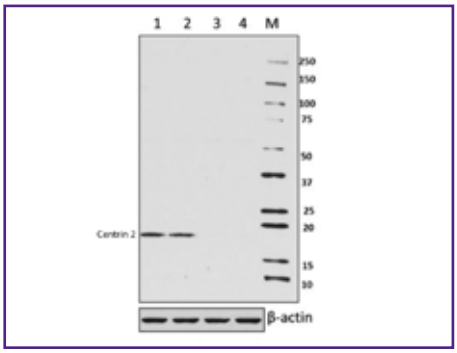
Specificity	M	P	CP
Eg5-Phosphorylated (Thr927)		•	
Flightless-I Protein	•		
H2A.X	•	•	
H2A.X Phospho (Ser139)	•		
Helix NP™ Blue			•
Helix NP™ Green			•
Helix NP™ NIR			•
KIN-28		•	
MAD2		•	
MCM3		•	
MCM5		•	
MCM6		•	
MCM7		•	
Ninein		•	
NOD1	•		
p27Kip1		•	
p53	•		
p97/VCP	•		
PARP	•		
Phospho-CAK (cdk7) (Ser164/ Thr170)		•	
PLK-1	•		
PLK-1 Phospho (Thr210)	•	•	
PRC1	•		
Prohibitin		•	
Propidium Iodide			•
PTEN	•		
Rad23B	•		
RCC1		•	
SMC1L1/SMC1		•	
Stathmin/Op18 Phospho (Ser16)		•	
TPX2	•		
TRF2	•		

H2A.X becomes phosphorylated on serine 139 after double-stranded DNA breaks. Phosphorylated H2A.X (γ-H2AX) promotes DNA repair and maintains genomic stability.



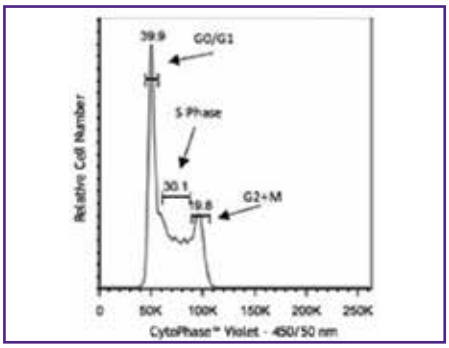
WB analysis of UV untreated and treated HeLa cells probed with Purified or Direct-Blot™ HRP anti-H2A.X Phospho (Ser139) antibody (clone 2F3). Purified anti-GAPDH antibody (clone Poly6314) was used as a loading control.

Centrins are small calcium binding proteins and the Centrin-2/Rad23B/XPC complex acts as an essential component of the nucleotide excision repair (NER) pathway.



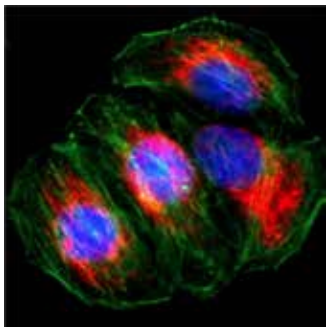
Total lysates from 293T (Lane 1), 20 nM scrambled siRNA (lane 2), 5 nM (Lane 3)and 20 nM (Lane 4) Centrin 2 siRNA treated 293T cells were probed with purified anti-Centrin 2 antibody (clone W16110A). Direct-Blot™ HRP anti-β-actin Antibody (clone 2F1-1) was used as a loading control.

CytoPhase™ Violet is a cell-permeant DNA-binding dye that can be used for flow cytometric analysis of cell cycle in live or fixed cells, and also for counterstaining the nuclei of cells in fluorescent microscopic imaging.



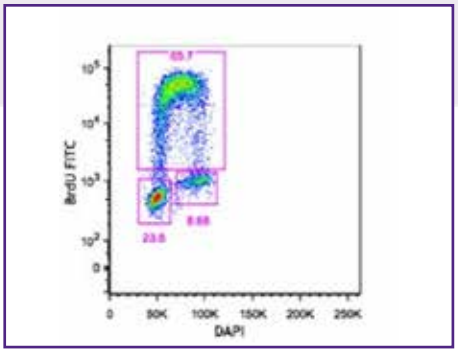
Ramos cells treated with 5 µM CytoPhase™ Violet dye for 90 minutes at 37°C. Cells were then acquired on a flow cytometer equipped with a 405 nm laser with a 450/50 bandpass filter.

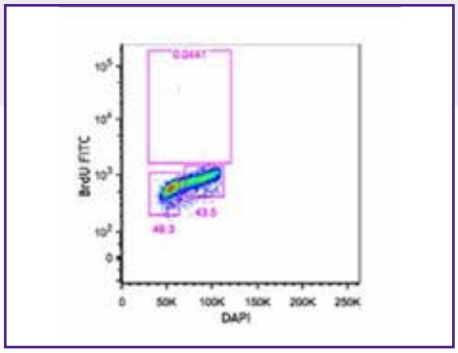
Cyclin-dependent kinase inhibitor 1A (CDKN1A), also known as P21 and Cip1, is a potent cyclin-dependent kinase inhibitor. CDKN1A binds to and inhibits the activity of cyclin-cyclin-dependent kinase2 or kinase4 complexes, thus functions as a regulator of cell cycle progression at G1.



HeLa cells were stained with purified anti-CDKN1A (clone W15115A) antibody, followed by Alexa Fluor® 594 secondary antibody (red), Alexa Fluor® 488 conjugated Phalloidin (green), and DAPI (blue).

BrdU is a Uridine derivative and a structural analog of thymidine, which can be incorporated into DNA during the S-phase of the cell cycle as a substitute for thymidine.



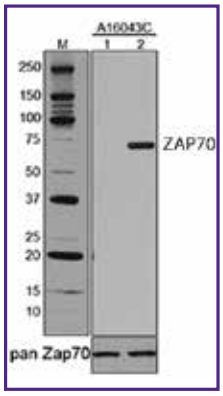


Ramos cells loaded with BrdU for 1.5 hours (left) or left as no load controls (right) stained using the Phase-Flow™ FITC BrdU Kit and DAPI.

Cell Signaling

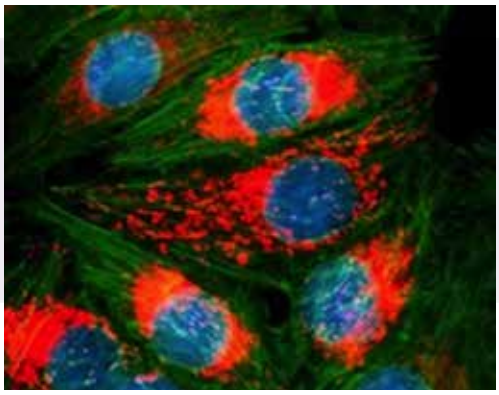
Cell signaling is the transmission and integration of signals from the outside to the inside of the cell. These signals are chemical, molecular, or mechanical cues that exert a specific effect on the cell. Cell proteins called receptors bind to signaling molecules (ligands) to initiate a physiological response, and different receptors are specific for different ligands. Once a receptor binds its ligand, it undergoes a conformational change, which in turn initiates a series of biochemical reactions within the cell. These intracellular signaling pathways (also known as signal transduction cascades) amplify the message using a network of enzymes and proteins. Enzymes in these signaling cascades typically modify the proteins they interact with in a process known as post-translational modification (PTM). Phosphorylation is one of the most common PTMs for regulating the activity and function of proteins. Since the ability of cells to correctly process signals is critical for their survival, errors in cell signaling typically cause diseases such as cancer, diabetes, and autoimmunity.

ZAP70 is a member of the Syk protein tyrosine kinase subfamily, expressed exclusively in T cells and NK cells that plays an essential role in T cell receptor (TCR) signaling in combination with the Src family kinases, LCK and FYN.



Jurkat cells treated without (Lane 1) or with (Lane 2) 2 mM H_2O_2 for 3 minutes were probed with purified anti-ZAP70 Phospho (Tyr493) antibody (Clone A16043C). Equal ZAP70 loading was confirmed using purified anti-ZAP70 antibody.

CARD9 is an adaptor protein that mediates the signaling downstream of C-type lectin receptors (CLRs), such as Dectin-1, Dectin-2, and Mincle.



HeLa cells were were intracellularly stained with anti-CARD9 Antibody (clone 13D9C60) followed by Alexa Fluor® 594 (red) conjugated goat anti-mouse IgG. Actin filaments were labeled with Alexa Fluor® 488 Phalloidin (green). Nuclei were counterstained with DAPI (blue).

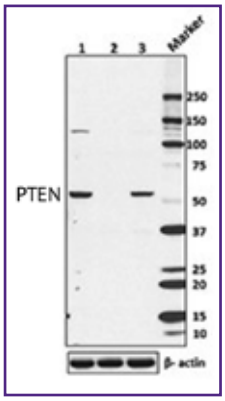
M = Monoclonal Ab
P = Polyclonal Ab

Specificity	M	P
β Catenin 1 (CTNNB1)	•	
β-Arrestin-1/2	•	
14-3-3 ζ/δ	•	
14-3-3 ε		•
ABCA3	•	
Aggrecan	•	
AKT1	•	
AKT2	•	
Akt Phospho (S473)		•
Allergin-1	•	
Amphiregulin	•	
Arginase I	•	
ATF4	•	
Aurora A (Aurora 2, AIK)		•
Aurora A (Aurora 2, AIK) phosph, (Thr288)		•
Aurora B	•	
B-Raf	•	
BTK Phospho (Tyr223)	•	
CAK (Cdk7) (Ser164/Thr170)		•
CaMKII	•	
CARD9	•	
CASK	•	
cdc2 (p34)	•	
CDK5	•	
c-Met	•	
c-REL	•	
DDX17 (p82)	•	
DDX17 (p82, p72)	•	
DDX58	•	
DNA-Pkcs Phosphorylated (Thr2609)	•	
Dopamine D3 receptor	•	
DR3 (TNFRSF25)	•	
DUX4	•	
E1 Ubiquitin Activating Enzyme	•	
EGF	•	
EGFR Phosphorylated (Tyr1068)		•
eIF2α		•
ER81		•
ERK1	•	
ERK1/2	•	
ERK1/2 Phospho (Thr202/Tyr204)	•	
ERK2		•
FOXP1	•	
FOXP3 Delta 2 (exon 2 deleted)	•	
Fyn	•	
GAB1	•	

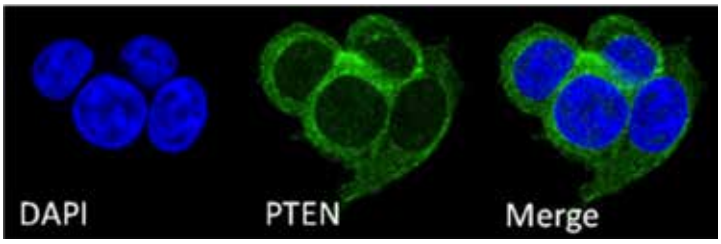
Specificity	M	P
GATA3	•	
GSK-3α	•	
Hamartin (TSC1)	•	
IκB-α	•	
IKBKB (IKKβ)	•	
IKKα	•	
IKKγ (NEMO)	•	
Lck	•	
LCK Phospho (Tyr505)	•	
LKB1 (STK11)	•	
Lyn	•	
MAPKAP1		•
MERTK	•	
mTOR	•	
MyD88	•	
NLRP 1, 2, 7, 12	•	
NOD1	•	
NOS2	•	
Notch1β	•	
NTAL	•	
NTAL (LAT2)	•	
P2RX7	•	
p38 MAPK Phospho (Thr180/Tyr182)		•
p90 Rsk		•
PERK Phospho (Ser713)		•
Phosphotyrosine	•	
PIK3R1	•	
PIR-A/B	•	
PKCα Phospho (T638)		•
PLCγ-1		•
PLK-1	•	
PLK-1 - Phosph, (Thr210)	•	•
Polyubiquitin (K63-linkage)	•	
PTEN	•	
PYK2	•	
RAPTOR		•
RPS6	•	
SH2D1A (SAP)	•	
SHIP-1	•	
SIK2	•	
SIRT1	•	
SMAD6	•	
SNAP-25	•	
SOCS3	•	
Sonic Hedgehog (SHH)		
SOX17	•	
SPHK1		

Specificity	M	P
STAT 1 , 3, 4, 5, 6	•	
STAT 2		•
STAT1 Phospho (Ser727)	•	
STAT3 Phospho (Ser727)	•	
STAT3 Phospho (Tyr705)	•	
STAT6 Phospho (Tyr641)	•	
STING	•	
SWAP70	•	
Syk	•	
Syntrophin	•	
TAB1	•	
TICAM-2 (TRAM)	•	
TIGIT (VSTM3)	•	
TIMP-1	•	
TRAF 1 , 3, 6	•	
TRIM	•	
TSC2	•	
Ubiquitin	•	
Wntless	•	
XCR1	•	
ZAP70	•	
ZAP70 Phospho (Tyr292)	•	
ZAP70 Phospho (Tyr319)	•	
ZAP70 Phospho (Tyr319)/Syk Phospho (Tyr352)	•	
ZAP70 Phospho (Tyr493)	•	

PTEN is a tumor suppressor with lipid and protein phosphatase activity that negatively regulates the PI3K/AKT pathway.

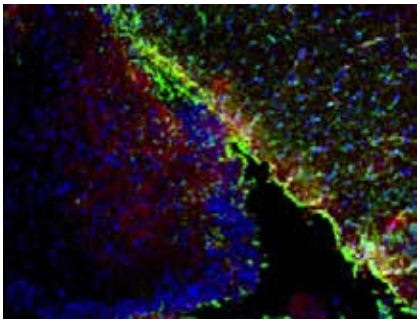


NIH3T3 cells (lane 1), PC3 cells (lane 2), and HeLa cells (lane 3) were probed with purified anti-PTEN antibody (clone 4C11A11) antibody. Purified anti-β-actin antibody (poly6221) was used as a loading control.



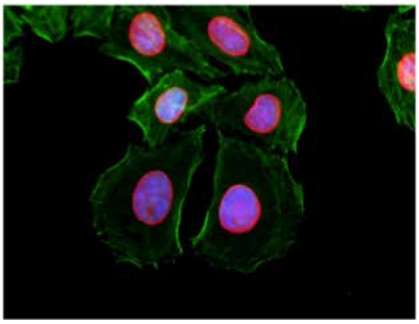
MCF7 cells were stained with purified anti-PTEN (4C11A11) antibody, followed by staining with DyLight™ 488 conjugated goat anti-mouse IgG (green) antibody. Nuclei were stained with DAPI (blue).

The Ca^{2+}/calmodulin (CaM)-dependent protein kinases (CaMKs) are multifunctional serine/threonine kinases whose activities are regulated through Ca^{2+} signaling.



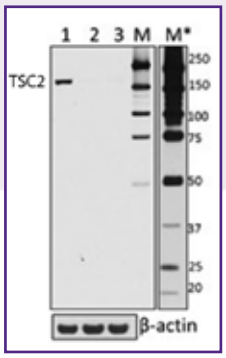
C57BL/6 mouse frozen brain section was stained with anti-CaMKII (clone 6G9) antibody and anti-GFAP (clone Poly28400) antibody followed by Alexa Fluor® 594 (red) conjugated goat anti-mouse IgG and Alexa Fluor® 488 (green) conjugated goat anti-mouse IgG. The nuclei were counterstained with DAPI (blue).

SMAD proteins are TGF-β signaling components that consist of receptor-regulated SMADs (SMAD1/2/3/5/9), a common SMAD (SMAD4), and inhibitory SMADs (SMAD6/7). SMAD6 is a negative regulator of TGF-β and bone morphogenetic proteins.



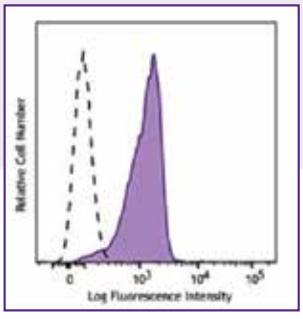
HeLa cells were intracellularly stained with SMAD6 Antibody (P85H3) and followed by Alexa Fluor® 594 goat anti-mouse IgG (red). Actin filaments were labeled with Alexa Fluor® 488 Phalloidin (green). Nuclei were counterstained with DAPI (blue).

Tuberous sclerosis 2 (TSC2) forms the tuberous sclerosis complex with TSC1 and functions as a crucial negative regulator of the Rheb/mTOR pathway.



HeLa (Lane 1), 5 nM (Lane 2), and 20 nM (Lane 3) TSC2 siRNA treated HeLa cells were probed with purified anti-TSC2 antibody. Direct-Blot™ HRP anti-β-actin Antibody was used as a loading control.

The Tyr641 residue of STAT6 is phosphorylated by Jak. Phosphorylated STAT6 forms homodimers, transclocates to the nucleus, and regulates transcription of target genes.

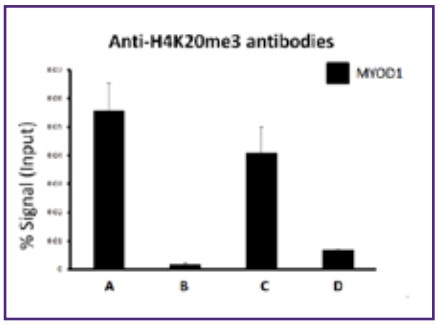


Human peripheral blood lymphocytes were stimulated with (filled histogram) or without (open histogram) recombinant human IL-4, permeabilized with True-Phos™ Perm Buffer, and intracellularly stained with STAT6 Phospho (Tyr 641) (clone A15137E) PE/Cy7.

Epigenetics and Transcriptional Regulators

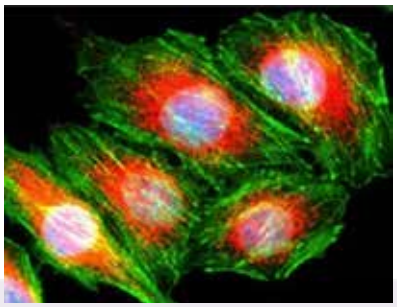
In eukaryotic cells, DNA is assembled into chromatin with repeating units of nucleosomes. Each nucleosome consists of eight core histone proteins, two each of H2A, H2B, H3, H4, which is wrapped with 147 base pairs of DNA. This complex forms the basis of the epigenetics landscape and is a dynamic system that undergoes multiple post-translational modifications (PTMs) and interacts with several regulatory proteins, including transcription factors to control gene expression. Common examples of PTMs are methylation, acetylation, phosphorylation, and ubiquitination that can alter chromatin structure. This, in turn, can dictate gene expression patterns in a cell by regulating the relative accessibility of transcription factors and the transcriptional machinery to the DNA.

Lysine N-methyltransferase 5C (KMT5C) is a Histone-lysine N-methyltransferase that specifically trimethylates Histone H4 Lysine 20 (H4K20). The more heavily methylated H4K20 becomes, the less transcription of the associated genes occurs.



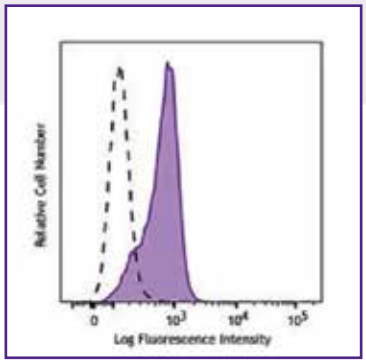
Chromatin Immunoprecipitation (ChIP) was performed with chromatin samples from HeLa cells with either A) Go-ChIP-Grade™ Purified anti-Histone H4 Trimethyl (Lys20) Antibody (clone 6F8-D9), B) equal amount of matched isotype Control Antibody (clone MOPC-21), or C) competitor's ChIP-grade Purified anti-Histone H4 Trimethyl (Lys20) Antibody and D) equal amount of matched Isotype Control Antibody as recommended by the manufacturer. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human MYOD gene regions.

IRF1 was the first identified member of the interferon regulatory transcription factor family, and is involved in the regulation of cell cycle progression, tumor suppression, and apoptosis.



HeLa cells were stained with anti-IRF1 Antibody (clone 13H3A44) followed by Alexa Fluor® 594 (red) conjugated goat anti-mouse IgG. Actin filaments were labeled with Alexa Fluor® 488 Phalloidin (green). Nuclei were counterstained with DAPI (blue).

Nanog is a homeodomain-containing transcription factor that is essential for early embryonic development.



NTERA-2 cells were intracellularly stained with anti-Nanog (clone 16H3A48) Alexa Fluor® 647 (filled histogram) or mouse IgG1, κ Alexa Fluor® 647 isotype control (open histogram).

M = Monoclonal Ab
P = Polyclonal Ab

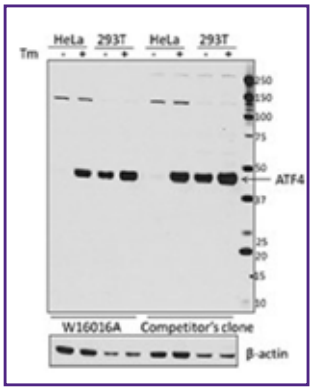
Specificity	M	P
Ahr	•	
Aiolos	•	
AND-1 (WDHD1)	•	
Ataxin-3	•	
ATF4	•	
ATF7	•	
BACH 1, 2	•	
BATF	•	
Bcl11b	•	
Bcl-6	•	
Blimp-1	•	
BRD4	•	
C/EBP b	•	
C/EBP b (2 isoforms C/EBP b, LAP)	•	
C/EBPa	•	
C2H2 zinc finger proteins	•	
c-Fos		•
c-MAF	•	
c-REL	•	
CRP	•	
Cystatin C	•	
DNMT1	•	
DNMT3B	•	
DUX4	•	
EGR2	•	
eIF-2a		•
eIF3D	•	
eIF3F		•
eIF3G	•	
EIF3I	•	
eIF3J		•
Elongin B	•	
Elongin C		•
EOMES	•	
EOS	•	
ER81		•
ETS2	•	
FOXA2	•	
FOXD3		•

Specificity	M	P
FOXO1	•	
FOXO3	•	
FOXP1	•	
FOXP3		•
FOXP3 Delta 2	•	
GAB1	•	
GATA3	•	
GCN5		•
Gli-1		•
Granzyme B	•	
Gre A	•	
Gre B	•	
HDAC1	•	•
HDAC 2, 3, 4, 7, 8	•	
HIF1-beta	•	
Histone H2B		•
Histone H3		•
Histone H3 (C-terminus)	•	
Histone H3 Dimethyl (Lys9)	•	
Histone Lysine Demethylase (NO66)	•	
Histone H3 Monomethyl (Lys9)	•	
Histone H3 Nonmethyl (Lys9)	•	
Histone H4 Monomethyl (Lys20)	•	
Histone H3.1 Phospho (Ser28)	•	
Histone H3 Trimethyl (Lys9) Antibody	•	
Histone H4 Trimethyl (Lys 20)	•	
HIV TAT	•	
HOXA3	•	
Hoxb-1		•
HRF		•
Ikaros	•	
IRF1, 2, 3, 4, 5,6, 7, 8, 9	•	
KIN-28		•
LEF1	•	
MAFB	•	
MBD1	•	
Nanog	•	
NFATc1, c2, c3	•	

Specificity	M	P
NFIL3	•	
NF-kappaB p100/p52	•	
NF-kB p50	•	
NF-kB p65	•	•
NF-κB p105/p50	•	
NF-κB p65	•	
NKX2-1	•	
NR4A2	•	
NR5A2	•	
Nur77	•	
NusA	•	
OCT 2, 4	•	
Pax-2		•
Pax-5	•	
Pax-6		•
PAX9	•	
POU2AF1	•	
PPAR-γ	•	
PLZF	•	
Prox1	•	
RAP30	•	
RARα		•
RBPJ	•	
RNA Polymerase beta	•	
RNA Polymerase beta Prime	•	
RNA Polymerase II	•	
RNA Polymerase II RPB1, 3, 4, 5, 6, 8,11	•	
RNA Polymerase TFIIB	•	
RNK	•	
RORg	•	
RORgt	•	
RORα	•	
RUNX1, 2, 3	•	
SATB1	•	
Sigma 32	•	
Sigma 54 (strain MG1655)	•	
Sigma 70 (strain MG1655)	•	
Sigma E	•	

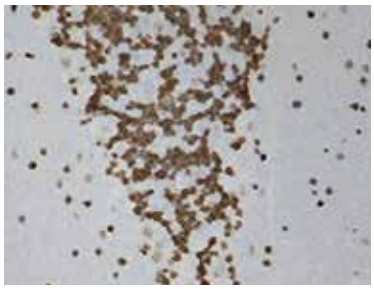
Specificity	M	P
Sigma F (strain MG1655)	•	
Sigma FecI	•	
Sigma S (Strain MG1655)	•	
Sir1	•	
Sir3	•	
SOX17	•	
SOX2	•	•
SOX2 (NH2 terminus)		•
Sp3		•
SP4	•	
SPI1	•	
Spt16 (FACT140 complex)	•	
SSRP1	•	
STAT1 Phospho (Ser727)	•	
STAT 1 , 3, 4, 5, 6	•	
STAT1 Phospho (Ser727)	•	
STAT3 Phospho (Ser727)	•	
STAT3 Phospho (Tyr705)	•	
STAT6 Phospho (Tyr641)	•	
TAL1	•	
Tata Binding Protein	•	
T-bet	•	
TCF1	•	
TCF1 (TCF7)	•	
TCF12	•	
TCF3	•	
TCF8	•	
TDRD3	•	
TFIIB	•	
Th-POK	•	
TICAM-1	•	
TIF1b	•	
TOX	•	
TTF-1	•	
XBP-1s	•	
ZBP-1	•	

ATF4 belongs to the family of basic zipper-containing proteins that regulate two important target genes: CHOP (transcription factor C/EBP homologous protein) and GADD34 (growth arrest and DNA damage–inducible 34).



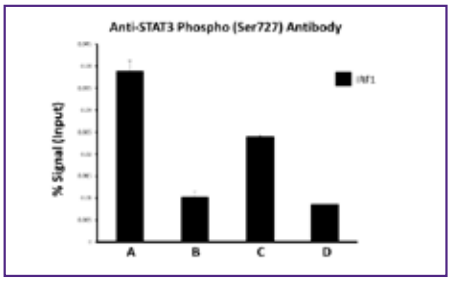
Non-treated and Tunicamycin (Tm) treated HeLa and 293T were probed with purified anti-ATF4 (clone W16016A) antibody or competitor's antibody. Direct-Blot™ HRP anti-β-actin (clone 2F1-1) antibody was used as a loading control.

Histone H3 can be modified by phosphorylation, acetylation, ubiquitination, ribosylation, and methylation, that regulate gene expression.

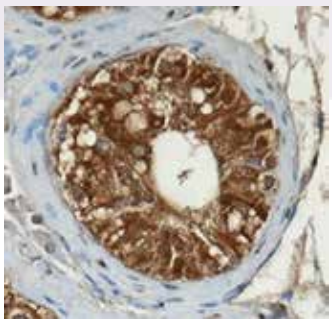


Staining of anti-Histone H3 Trimethyl (Lys9) Antibody (Clone 6F12-H4) on frozen rat brain.

Stat3 transcription factor is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation, and DNA binding. It is involved in the activation of genes required for cell growth and apoptosis.



Chromatin Immunoprecipitation (ChIP) was performed using chromatin samples from HeLa cells starved overnight and then treated with IL-6 with either A) Go-ChIP-Grade™ Purified anti-STAT3 Phospho (Ser727) Antibody (Clone A16089B), B) equal amount of Isotype Control Antibody, or C) competitor's ChIP-grade Purified anti-STAT3 Phospho (Ser727) Antibody and D) equal amount of matched Isotype Control Antibody as recommended by the manufacturer. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human IRF1 gene region.



Double homeobox 4, also known as DUX4, is a protein that has been reported to function as a transcriptional activator of paired-like homeodomain transcription factor 1 (PITX1).

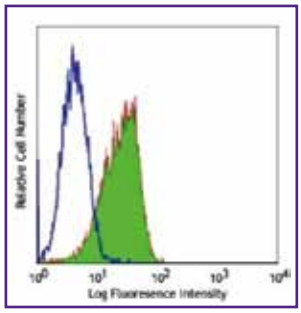
Staining of the seminiferous tubules in formalin fixed paraffin embedded human testis tissue using DUX4 (clone P2B1/DUX4).

Cell Adhesion and Extracellular Matrix

M = Monoclonal Ab
FP = Functional Proteins
E = ELISA
LP = LEGENDplex™

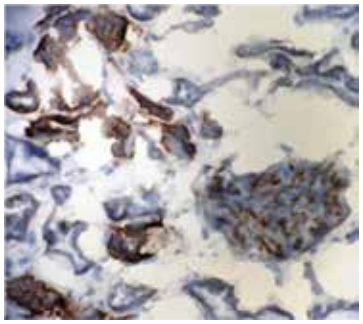
Cells adhere to each other and to the extracellular matrix through complex cellular structures that involve many proteins from receptor molecules to structural scaffolding proteins. These connections include focal adhesions (cell to matrix), adherens junction (cell to cell), and tight junctions (impermeable cell to cell connection). Transmembrane glycoproteins called cell adhesion molecules (CAMs) are integral to the formation of adhesions. Some CAMs are Ca^{2+} dependent such as cadherins, selectins, and integrins, whereas others such as N-CAM, which is expressed by a variety of cell types including most nerve cells, are Ca^{2+} independent. Cellular adhesion not only links cells, but can be involved in transmitting downstream signal as well.

E-Cadherin/CD324 is a member of the cadherin superfamily and is a calcium-dependent, transmembrane cell-cell adhesion glycoprotein.



Human colon carcinoma cell line (HT29) stained with purified CD324 (clone 67A4) (filled histogram), or purified mouse IgG1, κ (open histogram) followed by anti-mouse IgG FITC.

CD49b/ Integrin α2 associates with CD29 (β1 integrin) to form VLA-2, a collagen and laminin receptor on many cell types including activated T cells, neuronal cells and epithelial cells.

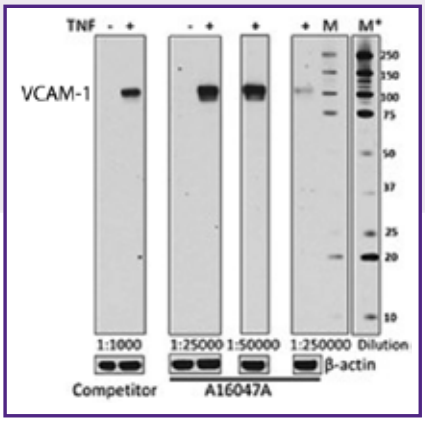


Staining of Integrin α2 (clone P1E6) on frozen normal human kidney tissue.

Specificity	M	FP	E	LP
ADAM22	•			
α-E-Catenin	•			
Aggrecan	•	•		
AMIGO-1	•			
β Catenin 1 (CTNNB1)	•			
Brevican	•			
Cadherin 11	•			
Cathepsin A	•	•		
Cathepsin B	•	•		
Cathepsin D	•	•		
Cathepsin E		•		
CD11a	•			
CD11a/CD18 (LFA-1)	•			
CD11b	•			
CD11c (Integrin αx subunit)	•			
CD18 (Integrin β2)	•			
CD29 (Integrin β1)	•			
CD31	•			
CD34	•			
CD41	•			
CD43	•			
CD43 Activation-Associated Glycoform	•			
CD47	•			
CD48	•			
CD49a	•			
CD49b (Integrin α2)	•			
CD49C (Integrin α3, URO-1)	•			
CD49D (Integrin α4)	•			
CD49e (Integrin α5)	•			
CD49f	•			
CD50 (ICAM-3)	•			
CD51 (Integrin αvβ5)	•			

Specificity	M	FP	E	LP
CD51/61	•			
CD54 (ICAM-1)	•	•		
CD58	•			
CD61	•			
CD62E (E-selectin)	•	•		
CD62P (P-selectin)	•			
CD62L	•			
CD62P (P-selectin)	•			
CD66a	•			
CD66a/c/e	•			
CD66C (CEACAM 6)	•			
CD73	•			
CD102 (ICAM-2)	•			
CD103 (αE Integrin)	•			
CD104	•			
CD105	•			
CD106 (VCAM-1)	•	•		
CD107b (LAMP-2)	•			
CD138	•			
CD144 (VE-Cadherin)	•			
CD146	•			
CD147 (EMMPRIN)	•			
CD151 (PETA-3)	•			
CD155 (PVR)	•			
CD156c (ADAM10)	•			
CD161f	•			
CD169 (Sialoadhesion)	•			
CD170 (Siglec-5)	•	•		
CD171	•			
CD172a (SIRPα)	•			
CD207 (Langerin)	•			
CD209a (DC-SIGN)	•			
CD227 (Muc-1)	•			

CD106/VCAM-1 is involved in cell adhesion and acts as a counter-receptor for VLA-4 (α4/β1 integrin) and LPAM-1 (α4/β7 integrin).

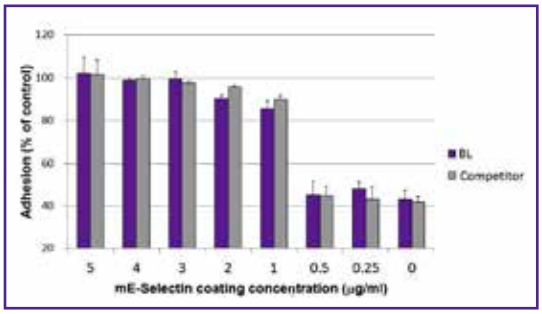


WB analysis of HUVEC and HUVEC treated with recombinant Human TNF-α probed with different concentrations of BioLegend's (clone A16047A) or competitor's anti-CD106 (VCAM-1) antibody, and Direct-Blot™ HRP anti-β-actin Antibody as a loading control.

Specificity	M	FP	E	LP
CD324	•			
CD324 (E-Cadherin)	•			
CD325 (N-Cadherin)	•			
CD326 (Ep-CAM)	•			
CD370 (CLEC9A, DNGR1)	•			
CD371 (CLEC12A)	•			
Claudin-1	•			
CLEC-2	•	•		
Clusterin	•	•		
Connexin 43	•			
EphA2	•	•		
Ephrin-A1	•	•		
ESAM	•			
FAK	•			
γ Protocadherin A (pan reactive)	•			
γ-protocadherin-B2	•			
Galectin-1		•		
Galectin-3	•	•		
Galectin-4		•		
Galectin-9	•	•		
HVEM	•	•		
ILK	•			
Integrin α4β7	•			
Integrin α9β1	•			
Integrin αV/β3	•			
Integrin β4	•			
Integrin β5	•			
Integrin β7	•			
LFA-1	•			
LPAM-1 (Integrin a4b7)	•			
Mac-2 (Galectin-3)	•			
MAdCAM-1	•			
MMP-1	•	•		

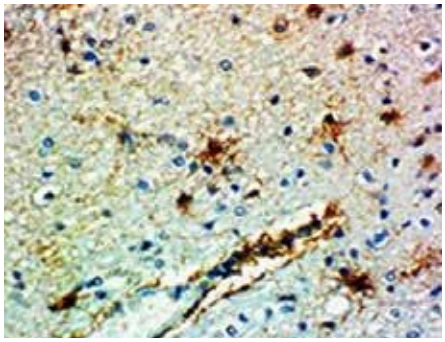
Specificity	M	FP	E	LP
MMP-2	•	•	•	•
MMP-3	•	•	•	
MMP-7		•		
MMP-8	•	•		
MMP-9	•	•	•	•
MMP-10		•		
MMP-12		•		
Neuroligin-1	•			
Neuroligin-3	•			
NrCAM	•			
Oligodendrocyte-myelin glycoprotein	•			
p120 Catenin	•			
Paxallin	•	•		
PNAd	•			
Podoplanin	•	•		
Siglec E		•		
Siglec H	•	•		
Siglec-3		•		
Siglec-9	•			
SALM2 (LRFN1)	•			
SynCAM4	•			
TIMP-1	•	•		•
TIMP-2	•	•		•
TIMP-3				•
URO-1	•			
Urokinase (uPA)	•	•		
VAP-1	•	•		
Vimentin	•			
Vitronectin	•	•		

E-Selectin, an adhesion molecule, belongs to the selectin/LECAM family and is only expressed in endothelial cells.

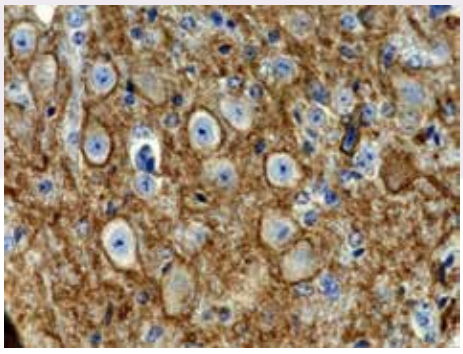


Immobilized recombinant mouse E-Selectin/CD62E protein induces 85-100% adhesion of U937 cells. BioLegend's protein was compared side-by-side to a competitor's equivalent product.

Connexins are a family of transmembrane proteins that assemble to form vertebrate gap junctions, thus also called Gap Junction Proteins.



IHC staining of purified anti-Connexin 43, 360-382 antibody (clone P2C4) on formalin-fixed, paraffin-embedded normal human brain tissue.



Brevican is a brain chondroitin sulfate proteoglycan, specifically expressed in the central nervous system, that plays a role in development and formation of the brain extracellular matrix and may be involved in malignancy of brain tumor cells.

IHC staining of anti-Brevican antibody (clone N294A/6) on formalin-fixed paraffin-embedded mouse brain tissue. Ultra Streptavidin (USA) HRP Detection Kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining.

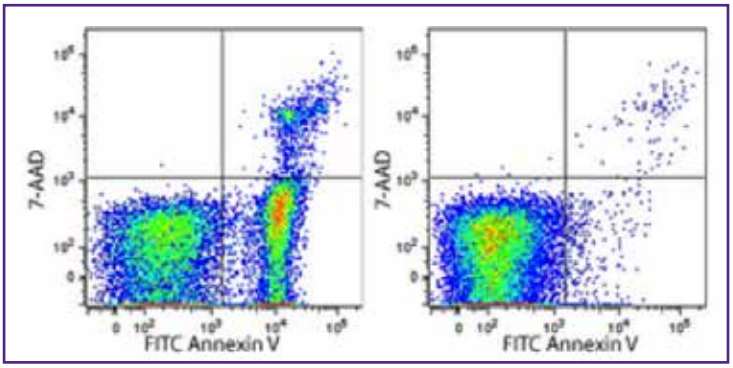
Cell Death

Apoptosis (also referred to as programmed cell death) is a naturally occurring process that leads to changes in cell morphology and ultimately cell death. It is a highly regulated and sequential process that can be initiated through one of two pathways - the intrinsic apoptosis pathway, where the cell kills itself because of factors such as stress and DNA damage, and the extrinsic pathway, where the cell kills itself because of external signals from other cells. Although both pathways induce cell death by activating caspases, the intrinsic pathway is mainly regulated by the Bcl-2 family of proteins, whereas the extrinsic pathway is under the control of members of the TNFR family of proteins which are the death receptors.

Unlike apoptosis, necrosis is an unregulated process of cell death that is caused by factors external to the cell or tissue, such as trauma, toxins, or infection. Cell death by necrosis is characterized by swelling of the cell, nuclear shrinkage, breakage of the nucleus into fragments, and plasma membrane rupture.

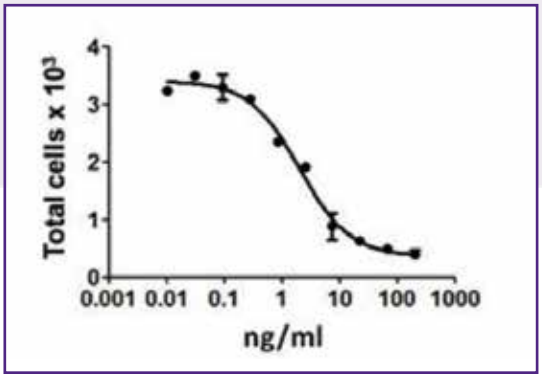
As cells die, whether by apoptosis or necrosis, impermeant nucleic acid stains, esterase activity-dependent probes, and mitochondrial respiration probes become useful tools to detect the live/dead status of the cells.

Annexin V (or Annexin A5) is a member of the annexin family of intracellular proteins that binds to phosphatidylserine (PS) in a calcium-dependent manner. Fluorochrome-labeled Annexin V can then be used to specifically target and identify apoptotic cells.



Human T-cell leukemia cell line, Jurkat, treated (left) or non-treated (right) with LEAF™ purified anti-human CD95 (clone EOS9.1) mAb, then stained with FITC Annexin V Apoptosis Detection Kit with 7-AAD.

Fas ligand (FasL, CD95L, TNFSF6) is a type-II transmembrane protein that belongs to the tumor necrosis factor (TNF) family. Its binding with its receptor induces apoptosis.



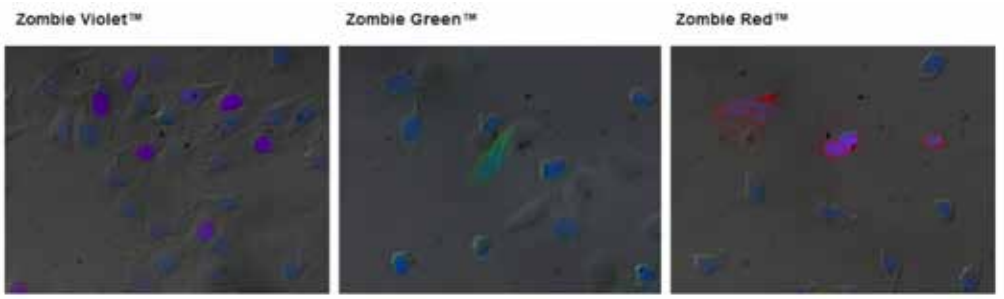
Apoptotic cell death induced by recombinant human FASL protein in Jurkat cells.

M = Monoclonal Ab
P = Polyclonal Ab
FP = Functional Proteins
E = ELISA
LP = LEGENDplex™
CP = Chemical Probes

Specificity	M	P	FP	E	LP	CP
7-AAD Viability Staining Solution						•
AIM2	•					
Akt Phospho (Ser473)		•				
AKT1	•					
AKT2	•					
Alix	•					
Annexin V			•			
Arginase I	•		•			
ASC (TMS-1)	•					
ATG17	•					
BAD	•					
BATF	•					
Bax	•	•				
BCL10	•					
Bcl-2	•					
Bcl-XS/L	•					
BID	•					
Bin1	•					
Bromodeoxyuridine (BrdU)	•					•
Calcein-AM						•
Calcein Violet-AM						•
CARD9	•					
Caspase 11						
Caspase-1, 2L, 7, 8, 9, 10, 12	•					
Caspase-3		•				
Cathepsin B, D	•		•			
CFSE Cell Division Tracker Kit						•
c-Myc	•					
CXCL12 (SDF-1β)						
Cytochrome c	•					
Daxx	•					
DcR1 (TRAIL-R3, CD263)	•					
DcR3	•					
DDX3X	•					
DDX5	•					
DR3 (TNFRSF25, TRAMP)	•					
DR4 (TRAIL-R1, CD261)	•					
DR5 (TRAIL-R2, CD262)	•					

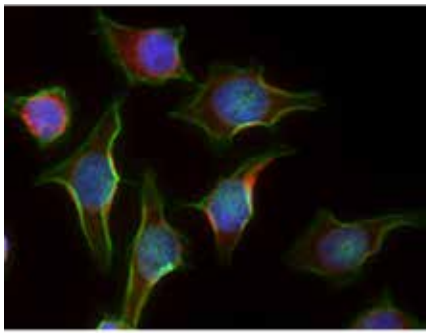
Specificity	M	P	FP	E	LP	CP
Fas (CD95)	•		•		•	
Fas-L (CD178)	•		•		•	
Galectin-1			•			
Galectin-3,9	•		•			
Galectin-4		•	•			
Granzyme A	•		•		•	
Granzyme B	•		•	•	•	
Granzyme K	•					
H2A.X	•	•				
H2A.X Phospho (Ser139)	•					
Helix NP™ Blue						•
Helix NP™ Green						•
Helix NP™ NIR						•
LDH-Cytox™ Assay Kit						•
MitoSpy™ Green FM						•
MitoSpy™ Orange CMTMRos						•
MitoSpy™ Red CMXRos						•
NLRP1	•					
NOD1 , 2	•					
P62	•	•				
PARP	•					
Perforin	•				•	
Propidium Iodide Solution						•
sTNF-RI (TNFRSF1A)	•					
sTNF-RII (TNFRSF1B)	•					
Survivin		•				
TL1A (TNFSF15)			•			
TNFα	•		•	•	•	
TNFβ	•		•		•	
TRAIL (TNFSF10)	•	•				
TWEAK (CD255)	•		•			
TWEAK receptor (Fn14, CD266)	•					
Zombie Dyes						•

Zombie dyes are a family of amine-reactive fluorescent dyes that is non-permeant to live cells but permeant to cells with compromised membranes. Thus, it can be used to assess live vs. dead status of mammalian cells in microscopy and flow cytometry.



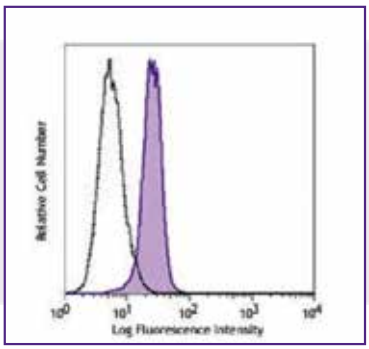
HeLa cells were treated with 20% EtOH and stained with Zombie Violet™ (magenta, left), Zombie Green™ (green, middle), or Zombie Red™ (red, right) and then fixed with 1% PFA. Nuclei were counterstained with DRAQ5™ (blue).

Cytochrome c initiates apoptosis by binding Apaf-1, which activates procaspase 9.



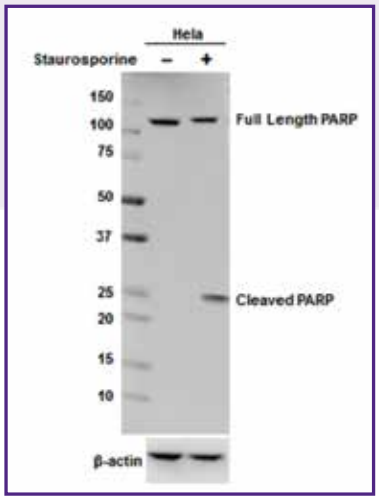
HeLa cells were stained with anti-Cytochrome c (clone 6H2.B4) Alexa Fluor® 594 overnight and Phalloidin Alexa Fluor® 488 (green). Nuclei were counterstained with DAPI (blue).

The relative levels of pro-apoptotic proteins such as Bax and anti-apoptotic proteins such as Bcl-2 determines whether cell death will occur following an apoptotic stimulus.



Human acute lymphoblastic leukemia cell line MOLT-4 were stained with Bax (clone 2D2) Alexa Fluor® 488 (filled histogram) or mouse IgG1, κ Alexa Fluor® 488 isotype control (open histogram).

PARP (Poly (ADP-ribose) polymerase) is a nuclear protein that functions in base excision repair, poly(ADP-ribosyl)ation of acceptor proteins involved in chromatin architecture and DNA metabolism, and participates in protein modification to enhance or repress transcription.



HeLa cells, untreated (-) or treated with Staurosporine (+, 1 µM, 8 hours) probed with anti-PARP (clone 5A5) antibody. Direct-Blot™ HRP anti-β-actin was used as a loading control.

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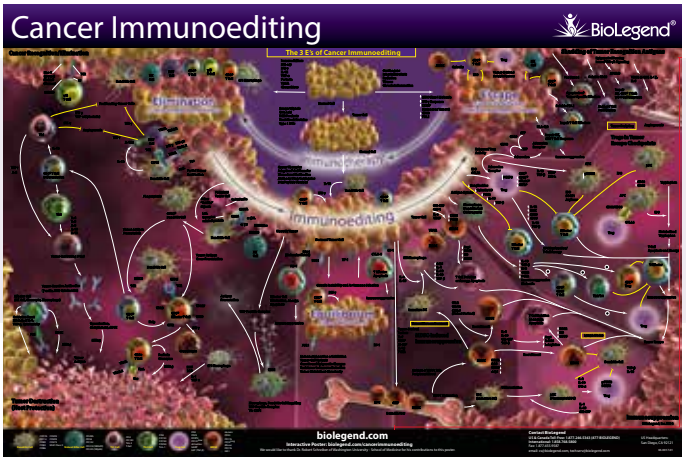
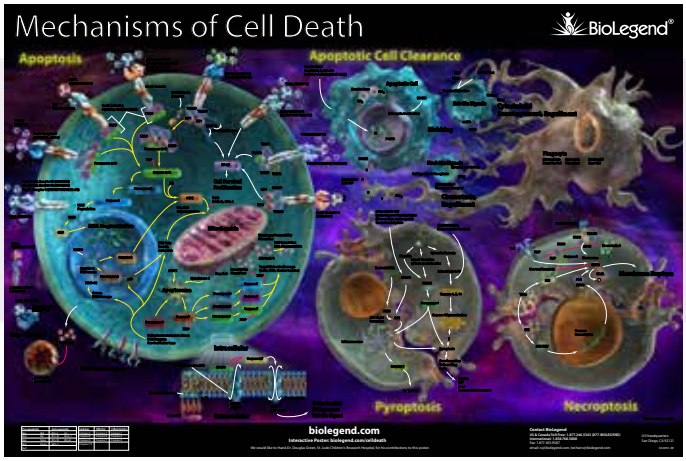
Cancer Immunoediting

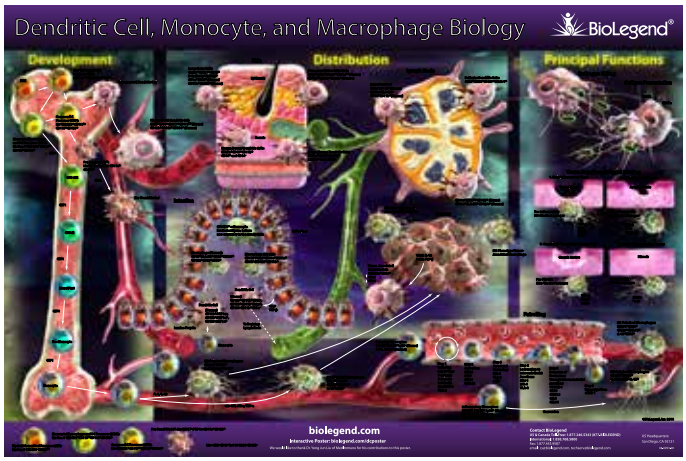


Mechanisms of Cell Death

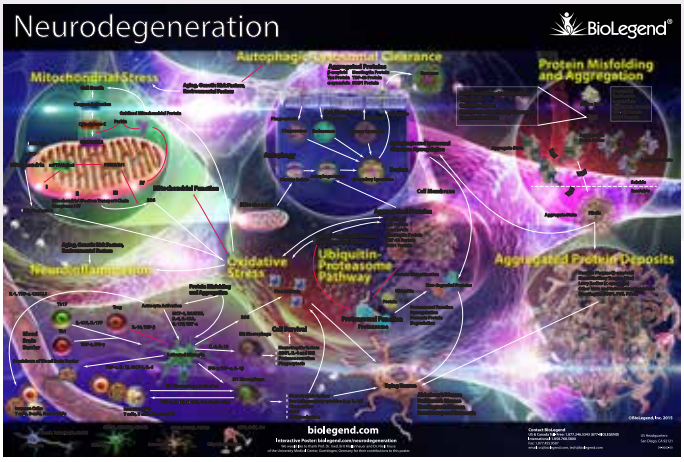


Innate Immunity

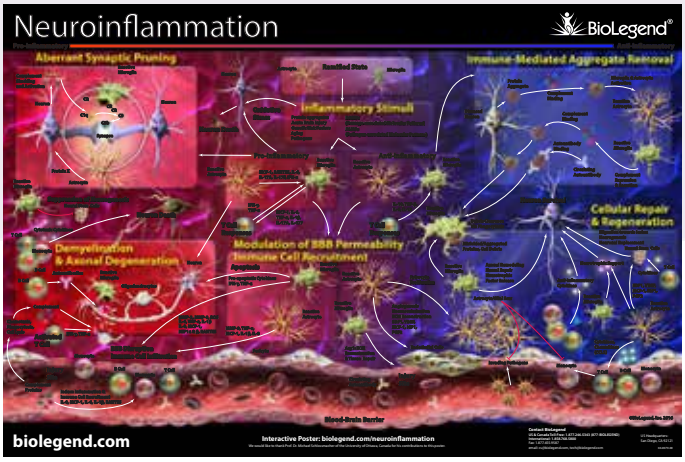
Dendritic Cells, Monocytes and Macrophage Biology



Neurodegeneration



Neuroinflammation



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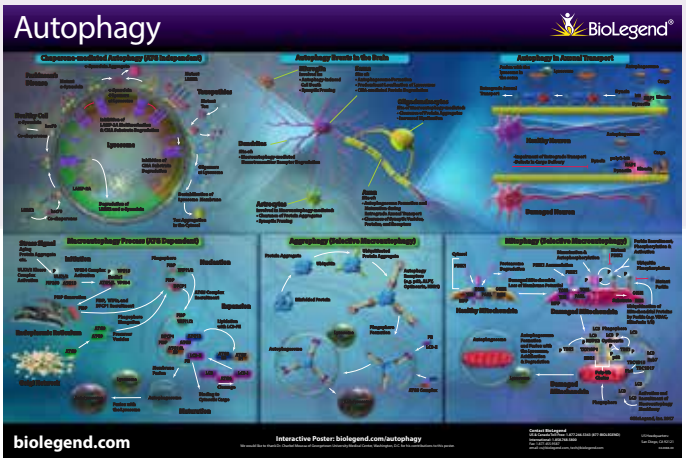
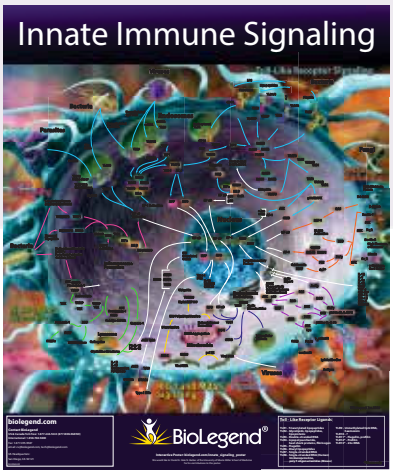
Autophagy



Innate Immune Signaling



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