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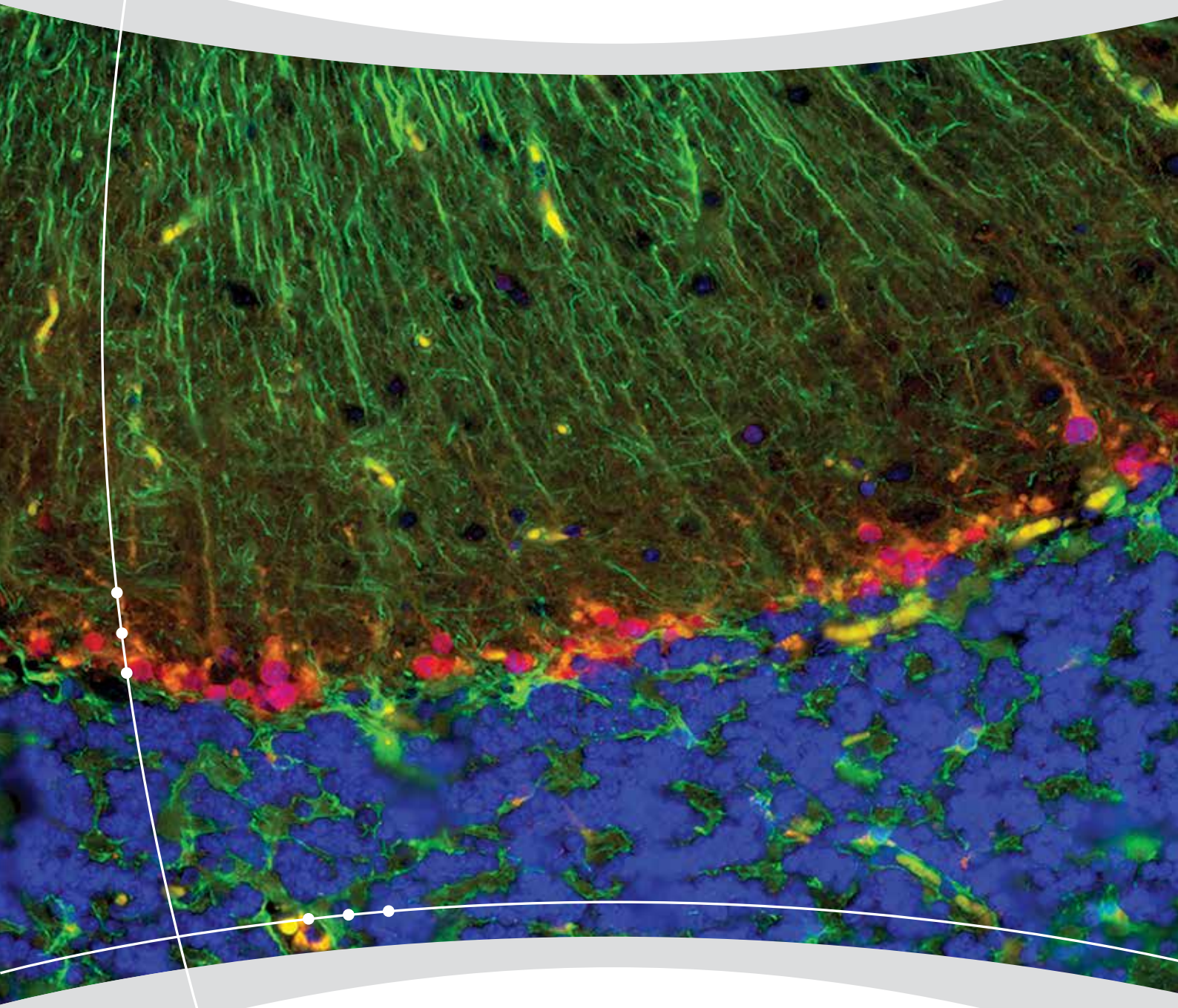
eBioscience

Visualize Expression

From Colorimetric to Immunofluorescence



Our mission is to enable the translation of biological knowledge into routine practice.

A pioneer in microarray technology and a leader in genomics analysis, Affymetrix now develops and provides innovative technologies that enable multiplex and parallel analysis of biological systems at the cell, protein, and gene level, facilitating the rapid translation of results into biology for a better world.

eBioscience, an Affymetrix company, is committed to developing and manufacturing high-quality, innovative reagents using an ISO-certified process. As a provider of more than 11,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality, and value.

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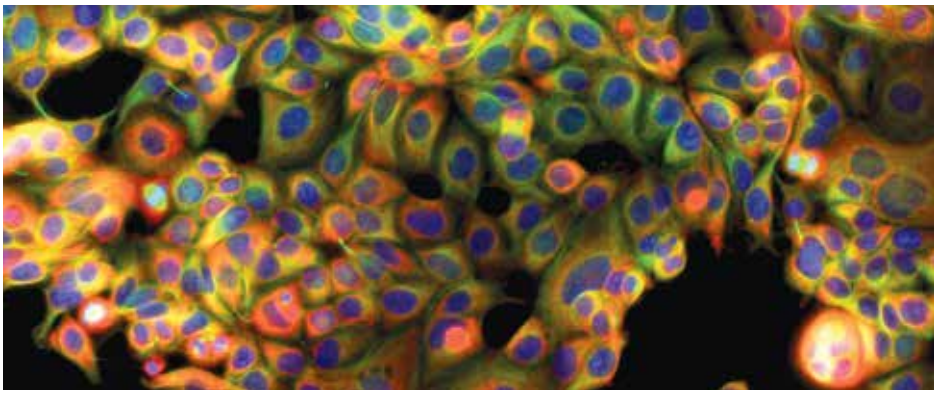
Overview

Microscopy is the most widely used imaging method to explore biological systems, in which immunocytochemistry (ICC), the localization of proteins within cells and immunohistochemistry (IHC), which reveals the abundance, distribution, and localization of biomarkers within a tissue, can be viewed directly. These techniques provide insight into cellular structure and mechanisms and are applicable for basic research, in addition to being indispensable in clinical settings. Target antigens may be evaluated using specific antibodies directly conjugated with enzyme or fluorophores, or indirectly using similarly labeled secondary antibodies and reagents.



Multicolor staining with eFluor® Organic Dyes

Multicolor staining of fixed, permeabilized MCF-7 cells using 5 ug/mL Anti-α Tubulin Alexa Fluor® 488 (green, cat. no. 53-4502), 10 ug/mL Anti-Human Cytokeratin 19 eFluor® 615 (red, cat. no. 42-9898) and DAPI (blue).

Antibodies for immunohistochemistry (IHC) and immunocytochemistry (ICC) are offered as purified or directly conjugated, having been optimized and validated for these applications. They are supported with protocols used by eBioscience R&D in addition to a comprehensive troubleshooting guide. When applicable, these antibodies are also tested for flow cytometry. The portfolio is focused on unique direct conjugates. Additionally, many purified and biotinylated primary antibodies are available for more traditional studies that use secondaries for fluorescence or colorimetric detection.

The extensive quality-control process begins with single-cell subcloning of hybridomas and isotype verification along with antibody production in tissue culture. Purity checks include gel electrophoresis and HPLC. Specificity is compared to isotype control staining in addition to other commercially available products. Conjugated antibodies are compared to unconjugated antibodies of the same clone and conjugated isotype controls. Fluorochrome to antibody ratios are optimized for each clone to achieve the best signal-to-noise.

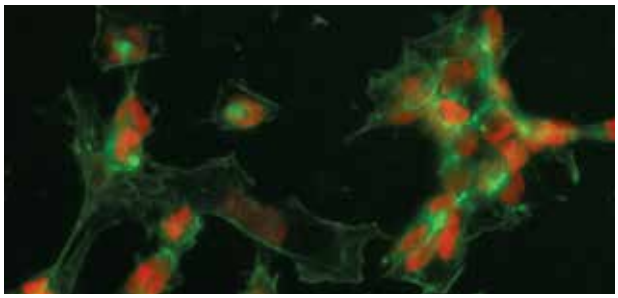
Final products must meet the quality-control specifications set for each particular product, which include a titer range required for detecting positive staining, proper localization of signal within the control tissue or cells, and comparison of multiple antibody lots as an internal positive control.

Direct and indirect detection methods

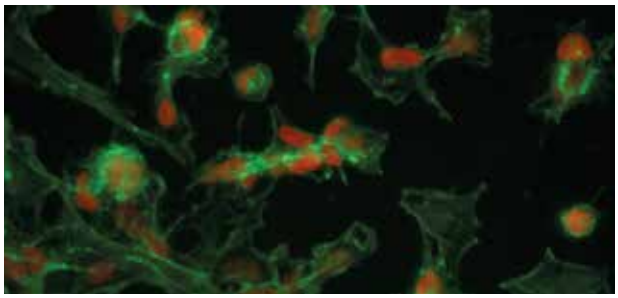
A comparison of multi-step and single-step approaches

Indirect staining involves two to three steps and provides greater signal amplification when the antigen is of low abundance; however there is potential for cross-reactivity due to the use of secondary reagents. Additionally, multiplexing capability is limited with the indirect method since there is a need to locate suitable primary antibodies raised in different species with different isotypes. Benefits of direct immunofluorescence include shorter sample staining times and the use of multiple antibodies raised in the same species.

Direct detection



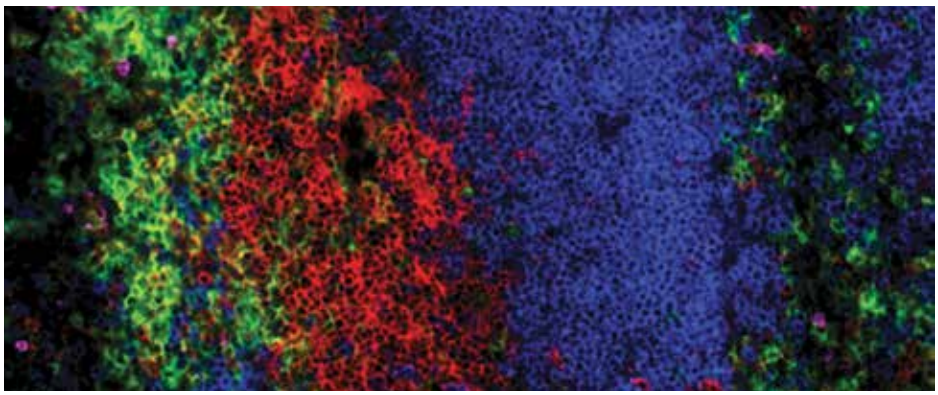
Indirect detection



5 ug/mL Anti-Human/Mouse Sox2 eFluor® 570 (left, red, cat. no. 41-9811) and 5 ug/mL Anti-Human/Mouse Sox2 Purified, (cat. no. 14-9811) followed by Anti-Rat IgG2a Biotin and Streptavidin eFluor® 570 (right, red, cat. no. 41-4317). Actin filaments are stained with Phalloidin eFluor® 520 (green, cat. no. 59-6559).

Multiplexing with directly conjugated antibodies

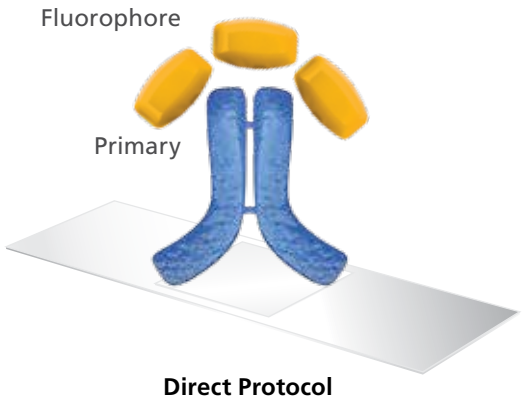
Detection of immune cells in frozen mouse spleen using Anti-Mouse CD4 eFluor® 570 (red, cat. no. 41-0042), Anti-Mouse CD11b Alexa Fluor® 488 (magenta, cat. no 53-0112), Anti-Mouse CD11c eFluor® 615 (green, cat. no. 42-0114), and Anti-Mouse CD45R (B220) eFluor® 660 (blue, cat. no. 50-0452).



Direct staining

eBioscience is known for innovative product designs and novel research tools. We provide a broad offering of antibodies directly conjugated to fluorophores that are ideal for fluorescent imaging. With our commitment to high quality and performance, all products are QC-validated in their intended application(s). Therefore our direct conjugates enable you to confidently and easily perform multicolor immunostaining with the following advantages:

- **Simple and fast** - evaluate multiple antigens in just one antibody staining step
- **Cost effective** - no need for additional secondary antibodies or detection reagents
- **Best for multiplexing**

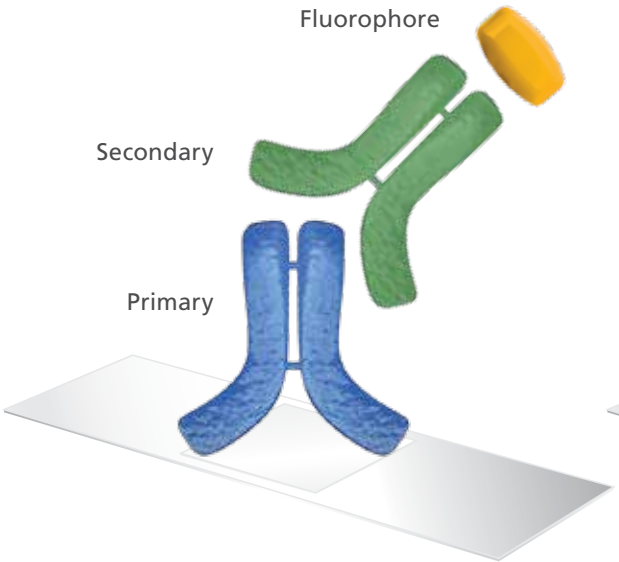


Direct Protocol

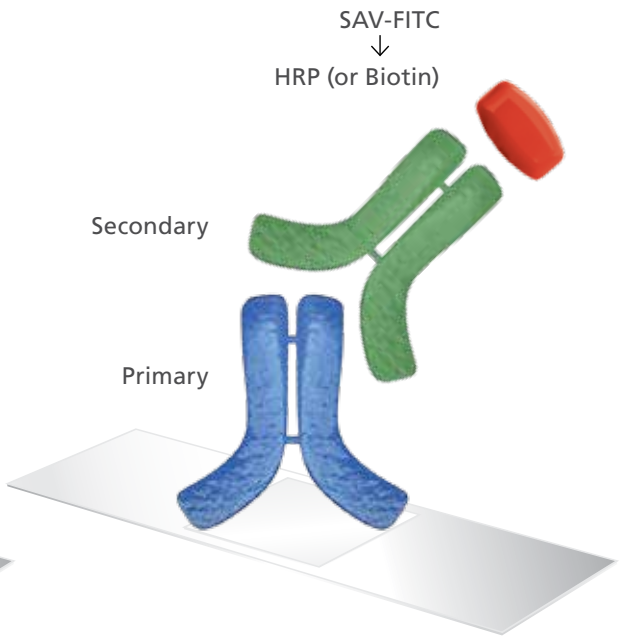
Indirect staining

Indirect staining involves multiple steps and is ideal for signal amplification when the antigen of interest is of low abundance. Purified (unconjugated) or biotin antibodies validated for use in microscopy are suitable for 2- and 3-step staining. Choosing reagents in this format offers the following advantages:

- **Increased signal amplification** - good for low-abundance antigens
- **Flexibility** - use with a variety of detection reagents



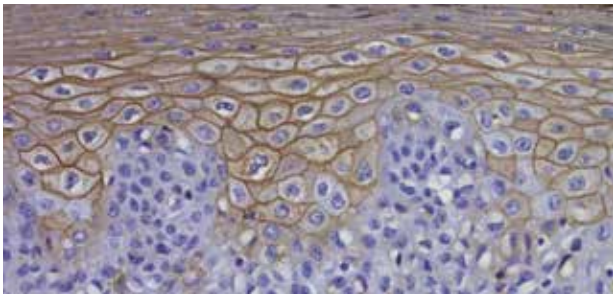
2-Step Protocol



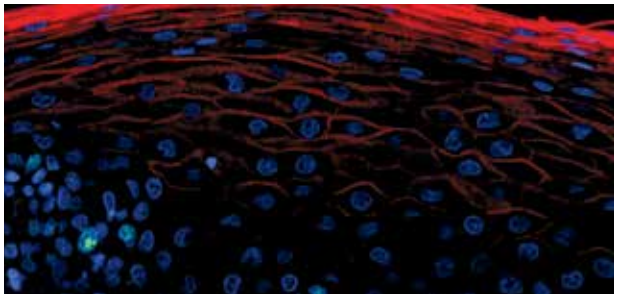
3-Step Protocol

Colorimetric or immunofluorescent staining

When your sample is precious and many targets need to be visualized simultaneously, staining multiple proteins simultaneously provides an opportunity to do more with less, particularly when direct conjugates are used. Colorimetric staining is a great choice when purified antibodies to your target of interest are the only option available.



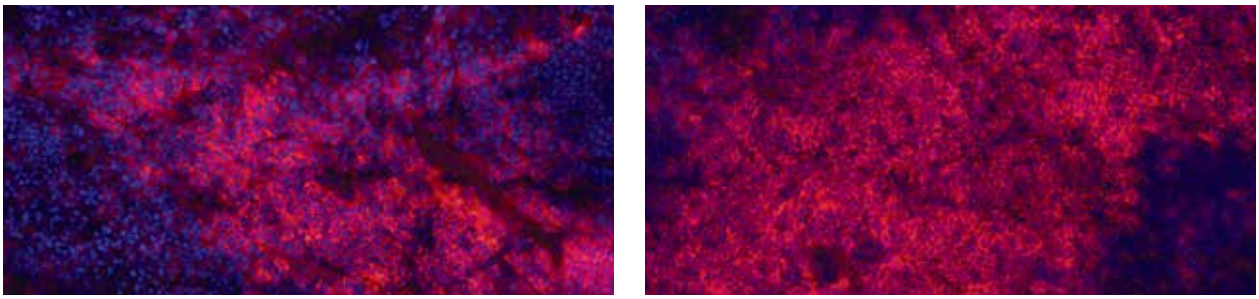
Visualization of Cutaneous Lymphocyte Antigen expression in formalin-fixed paraffin embedded human tonsil using Low-pH Antigen Retrieval Solution (cat. no. 00-4955) and 10 ug/mL Anti-Human/Mouse Cutaneous Lymphocyte Antigen (CLA) Purified (cat. no. 14-9857) followed by Anti-Rat IgG Biotin (cat. no. 13-4813), Streptavidin-HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.



20 ug/mL Anti-Human/Mouse Cutaneous Lymphocyte Antigen eFluor® 660 (red, cat. no. 50-9857). 5 ug/mL Anti-Human Ki-67 eFluor® 570 (green, cat. no. 41-5699). Nuclei are stained with DAPI (blue). Colocalization of Ki-67 and DAPI in nuclei appears aqua.

Comparable performance to Alexa Fluor® dyes

eFluor® is the eBioscience brand of fluorochromes for the labeling and detection of biomolecules. eFluor® Organic Dyes are a proprietary line of fluorescent dyes within the eFluor brand engineered for superior optical performance and detection, providing excellent brightness, resolution, and photostability.

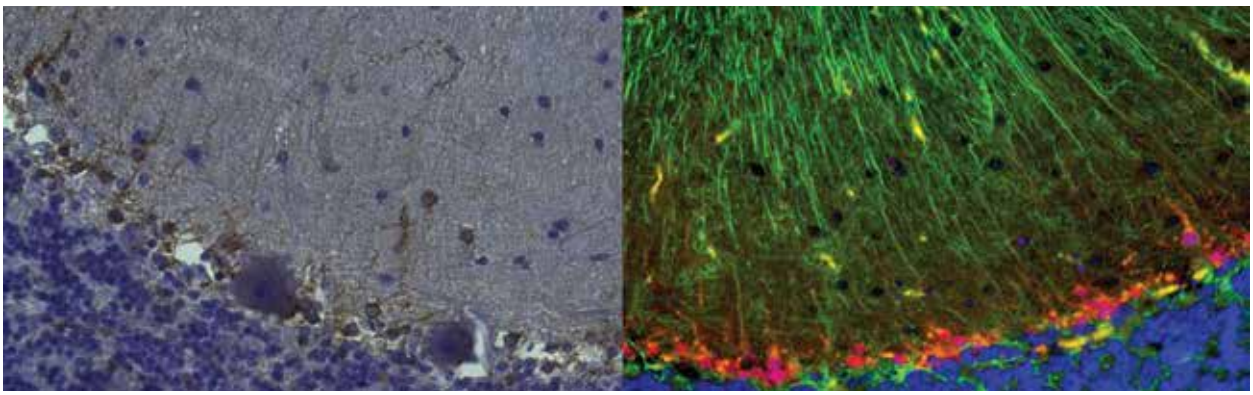


eFluor® 660 – Excellent specificity and photostability; an Alexa Fluor® 647 alternative

Direct conjugate staining of frozen mouse spleen using Anti-Mouse CD4 eFluor® 660 (left, cat. no. 50-0041) or Anti-Mouse CD4 Alexa Fluor® 647 (right). Nuclei were stained with DAPI (blue).

See cells and tissues differently: Use a multidimensional approach

Immunochemistry and *in situ* hybridization (ISH) when used in combination becomes a powerful research tool for characterizing cells or tissues to determine levels and spatial localization of transcript or mRNA and protein expression. *In situ* hybridization (ISH) allows for specific localization of nucleic acid targets in fixed and frozen tissues or cells, while immunohistochemistry (IHC) and immunocytochemistry (ICC) can be used to validate expression of antigens encoded by transcripts, which have been identified as being present in the sample of interest. Both assays provide multiplexing capabilities, either through the use of multiple target and detection probes or via directly conjugated antibodies.



From single detection to multiplexing

Immunohistochemistry of formalin-fixed paraffin embedded human cerebellar tissue using colorimetric visualization (left) or immunofluorescence (right). Left image shows staining using Anti-Human 10-formyltetrahydrofolate dehydrogenase (ALDH1L1) Purified (cat. no. 14-9595) followed by 1 ug/mL Anti-Mouse Biotin, Streptavidin-HRP, and DAB visualization. Nuclei are counterstained with hematoxylin. Right image shows staining using 10 ug/mL Anti-Human/Mouse Sox2 eFluor® 660 (red, cat. no. 50-9811, an Alexa Fluor® 647 alternative), 10 ug/mL Anti-Human 10-formyltetrahydrofolate dehydrogenase (ALDH1L1) eFluor® 570 (orange, cat. no. 41-9595), and 10 ug/mL Anti-Glial Fibrillary Acidic Protein (GFAP) Alexa Fluor® 488 (green, cat. no. 53-9892). Nuclei are stained with DAPI (blue). Colocalization of Sox2 and DAPI in nuclei appears pink.

Using nucleic acid probes in preserved, fixed, and frozen histologic specimens, enables the ISH assay user to visualize gene expression information in the context of tissue/cell morphology. In the past, quantitation of *in situ* gene expression at the RNA (mRNAs or non-coding RNA) level had limited utility due to low sensitivity of non-radioactive formats, complicated work flows, and the inability to simultaneously multiplex. However, recent advances in the amplification steps of ISH assays have eliminated the need for working with radioactive probes and have improved the feasibility of detecting multiple transcripts within the same sample.

In situ hybridization

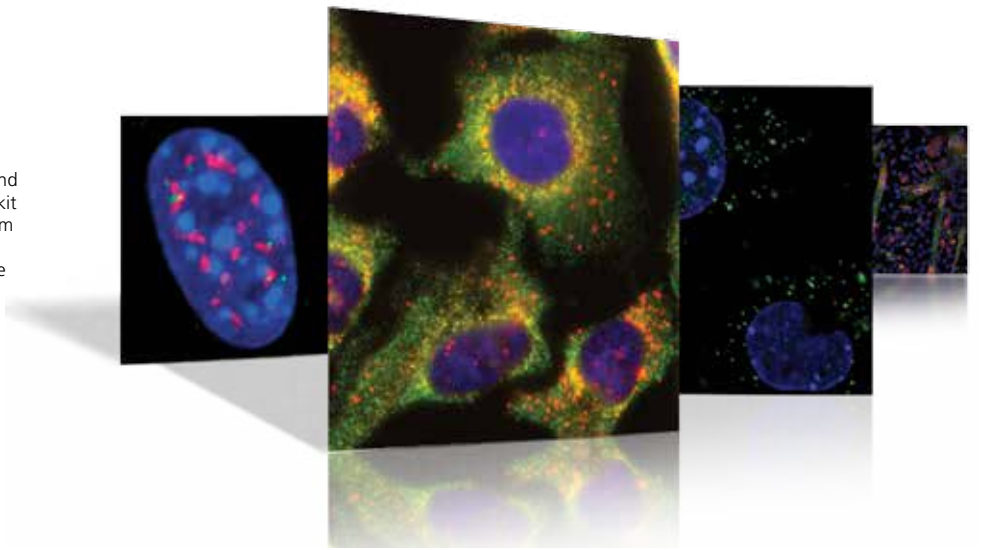
When you are interested in a specific mRNA, non-coding RNA or the antibody of interest is commercially unavailable, RNA probes enable your research to continue.

ViewRNA™ Assays for RNA *in situ* visualization provide:

- High specificity
- Simple protocol
- Sensitive single-molecule RNA detection
- Custom probe synthesis in less than 1 week

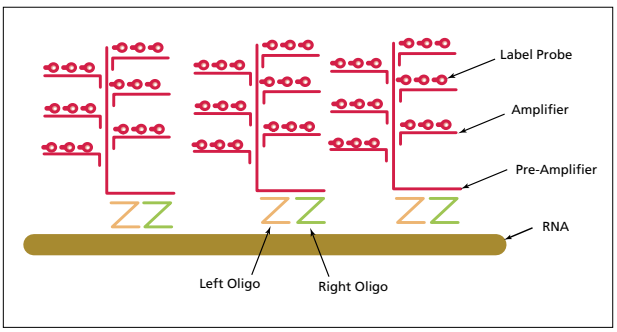
Out-of-the-box solution

RNA ISH assays are fast, easy, and reliable. Offered as a complete kit with the freedom to choose from an extensive catalog of probes or order custom ones, which are available in less than one week.



High sensitivity and specificity achieved with bDNA technology

ViewRNA Assays are based on patented branched DNA (bDNA) signal amplification technology. These novel RNA *in situ* hybridization (ISH) assays, enable localization and visualization of RNA in cells and tissues using singleplex or multiplex assay formats. ViewRNA Assays offer sensitive single-molecule RNA detection with virtually no background.



Probe set: Mixture of oligos that bind to a specific target sequence. Typical probe sets contain 20 oligo pairs.

Oligo pairs: Pair of adjacent oligos in a probe set. Each pair binds one PreAmp molecule.

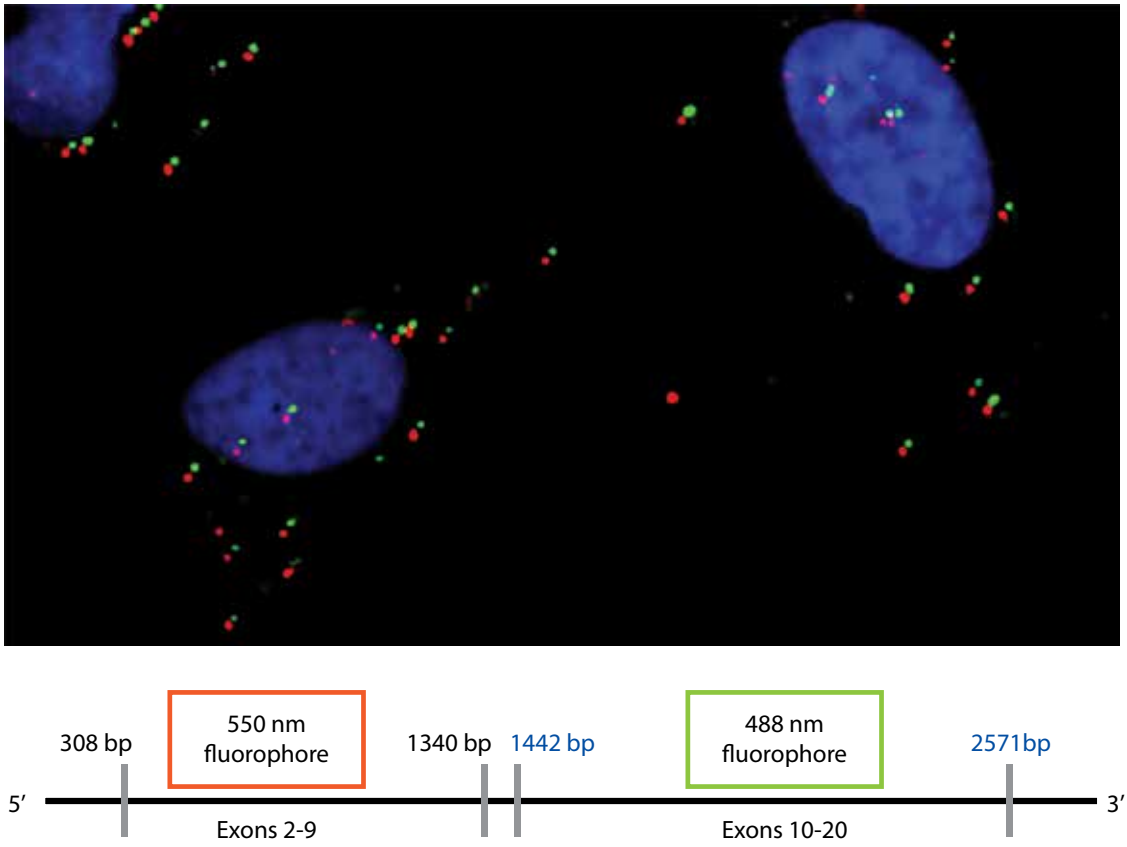
PreAmplifier (PreAmp): DNA molecule that interacts with Target Probe Set and Amplifier. Each PreAmp binds 20 Amps.

Amplifier (Amp): DNA molecule that interacts with PreAmp and Label Probe (LP). Each Amp binds 20 LPs.

Label Probe (LP): Oligo-conjugated with fluorescent dyes.

Branch/tree: Fully assembled structure of PreAmp, Amp, and LP, providing a 400-fold signal amplification with one oligo pair.

Single-transcript sensitivity



Specific, single-transcript sensitivity of the multiplex fluorescence RNA *in situ* hybridization assay.

Each set of red and green dots corresponds to a single copy of HER-2 mRNA. Nuclei (blue) are stained with DAPI. One dot is equivalent to one target molecule. Two Probe Sets were designed to target different regions of the ErbB2 (HER-2) mRNA. One Probe Set targeted the region from exons 2-9 (550-fluorophore) and the other Probe Set targeted the region from exons 10-20 (488-fluorophore). The image was captured with a slight offset to enable visualization of both signals. If one dot is equivalent to detection of one target, one would expect to see pairs of red and green dots as is evident in the image.

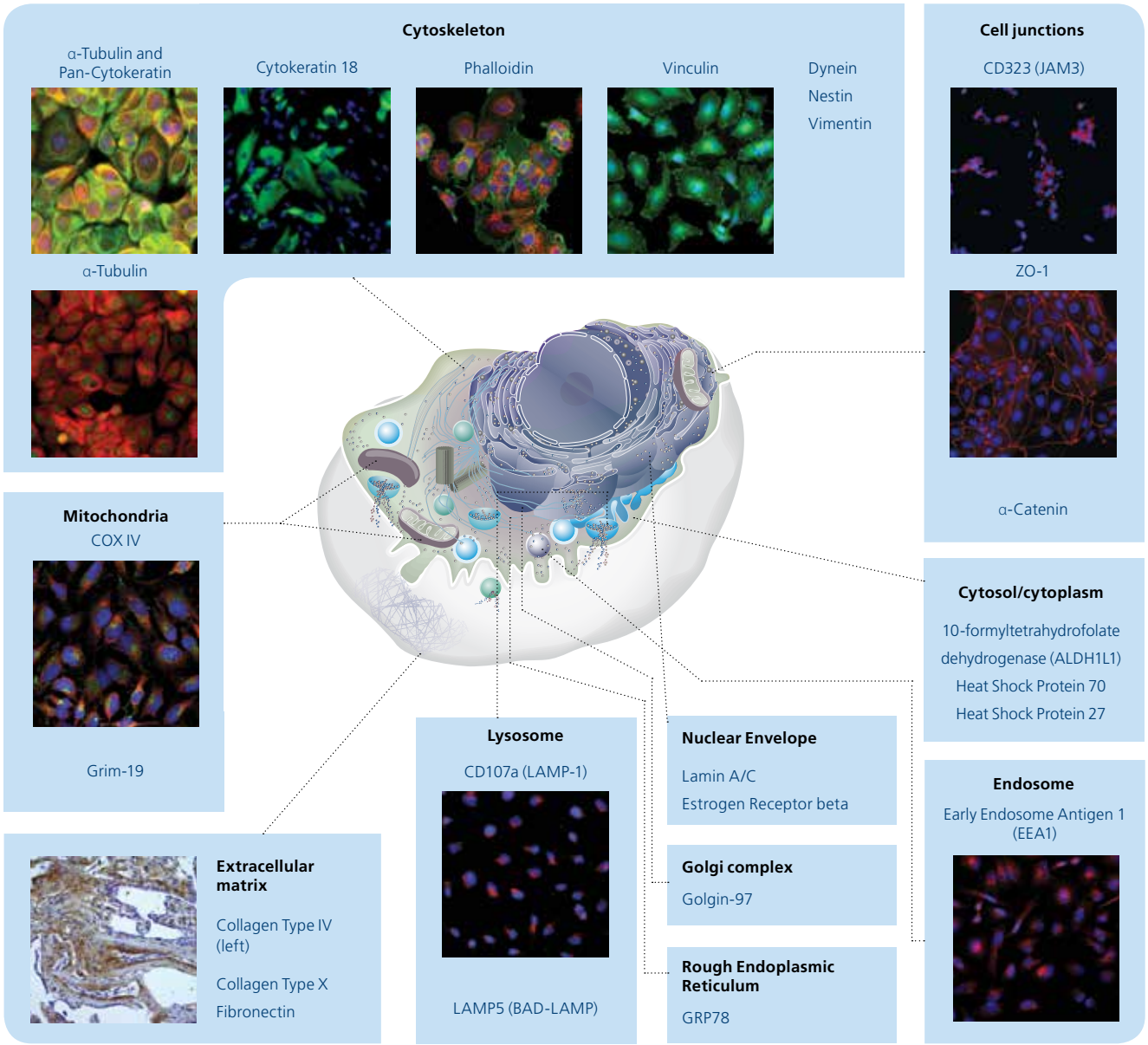
2

Cell structure

Eukaryotic cells are characterized by a nucleus containing DNA and numerous types of organelles with various functions. Organelles serve to localize activity (for example, energy production, regulation of protein synthesis, and modification) or movement of materials in and out of the cell. The cytoskeleton provides structural support to the cell, which is important for cell division, movement of organelles, cell morphology, and potential motility.

Cell structure and function		
Cell Nucleus	**Description**	**Function**
Nucleus	Contains nucleolus and chromosomes	Maintain integrity of genes Control cellular activity through regulation of gene expression
Nucleolus	Consists of rDNA, RNA and protein	Ribosomal RNA synthesis Ribosomal subunit assembly
Chromosomes	Condensed chromatin comprised of gDNA, protein, and RNA	Compact storage of genomic DNA that can be transcribed or replicated and passed on to daughter cells
Cytoplasmic Organelle	**Description**	**Function**
Plasma membrane	Cell membrane	Regulates movement of cell material Maintains cell shape Communicates with cells
Endoplasmic Reticulum (ER)	Interconnected network of flattened, membrane-enclosed sacs (cisternae) that are continuous with the outer membrane of the nuclear envelope	Synthesizes lipids Modifies proteins
Smooth ER	Smooth surface without ribosomes	Lipid biosynthesis
Rough ER	Ribosomes cover surface	Protein synthesis
Ribosomes	RNA and protein granules	Synthesizes polypeptides
Golgi complex	Stacked, flat membranes	Modifies proteins Packages and sorts proteins Lysosome formation
Lysosomes	Membranous sacs	Contains enzymes to break down materials, secretions, and waste Isolates harmful material from the rest of the cells in acidic environment Catabolism of fatty acid chains
Vacuoles		
Peroxisomes		
Mitochondria	Sac containing two membranes	Cellular respiration
Cytoskeleton	**Description**	**Function**
Microtubules	Hollow tubes forming subunits of tubulin	Structural support Organelle movement Cell division
Microfilaments	Solid, rod-like structures comprised of actin	
Intermediate filaments	Tough protein fibers	Strengthens cytoskeleton Stabilizes cell shape
Centrioles	Hollow cylinders near nucleus Contains microtubules	Anchors and organizes microtubule formation
Cilia	Fine, short projections extending from cell surface made from microtubules	Motile or primary cilia move cell itself or material along cell surface
Flagella	Long projections made from microtubules and covered by plasma membrane	Cell locomotion

Visualizing cell structure with organelle-specific antibodies



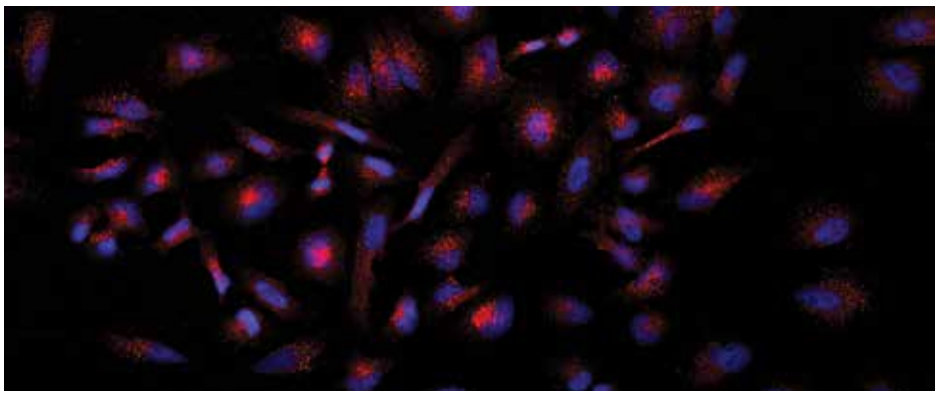
Understanding the sub-cellular location of proteins through co-localization studies provides information on the role they play in cellular processes. The cell's organelle markers can be categorized as membrane bound, which are involved in metabolic processes and include the nucleus, mitochondria, endoplasmic reticulum, Golgi complex, and endosomes or macromolecular complexes, which lack a membrane but are involved in cellular functions such as ribosomes, cytoskeleton, nuclear envelope, nucleolus, and centromeres. eBioscience continues to expand the organelle marker portfolio for characterization and identification, having recently launched Early Endosome Antigen 1, β-Tubulin Class III, Collagen 10, α-Catenin, and Ki-67 as purified antibodies, with new formats launching weekly.

Defining cell structures

Early endosome antigen 1 (EEA1) is a hydrophilic peripheral membrane protein involved in trafficking, found in the cytosol, and present on early endosome membranes. EEA1 is a dimer that binds phospholipid vesicles containing phosphatidylinositol 3-phosphate and localizes to early endosomes via a cysteine-rich zinc-finger-like FYVE domain, interacting with Rab5-GTP, syntaxin 6, and syntaxin 13 (SNARES). EEA1 is required for endocytic membrane docking and fusion, which allows for the recycling of receptors from the plasma membrane or their delivery to lysosomes for degradation. In neurons, early endosomes are involved in the recycling of neurotransmitter receptors. Autoantibodies to EEA1 have been found in patients with neurological deficits, which results in enhanced excitatory synaptic transmission.

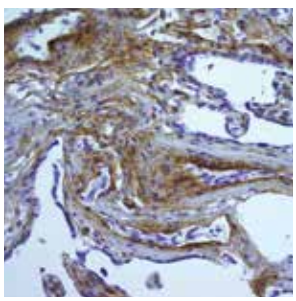
EEA1 present on early endosome membranes

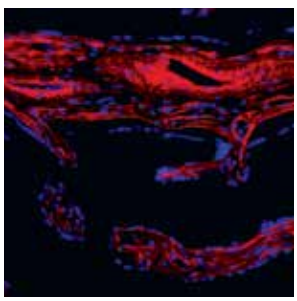
Early endosomes are visualized with ICC staining of formaldehyde-fixed and permeabilized HeLa cells using 1ug/mL Anti-Early Endosome Antigen 1 (EEA1) Purified (cat. no. 14-9114) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).



Collagen is an abundant, ubiquitous protein with approximately 20 genetically different types identified in the extracellular matrix (ECM). Collagen Type IV, VIII, and X form a mesh-like network of homotrimeric short-chain collagen. Collagen Type IV plays an important structural role in basement membrane formation and maintenance. Disruption of basement membranes is a significant marker of pathology during angiogenesis, tumor growth, and metastasis. Collagen Type X is primarily expressed during bone growth and development with limited expression in normal adult tissues. Expression is localized to the peri- and extracellular matrix of hypertrophic chondrocytes during endochondral ossification, playing a role in cartilage mineralization. Collagen Type X is elevated in many tumor types with expression localized in the vasculature. Mutations to the gene COL10A1, which encodes human Collagen Type X, is associated with Schmid-type metaphyseal chondrodysplasia.

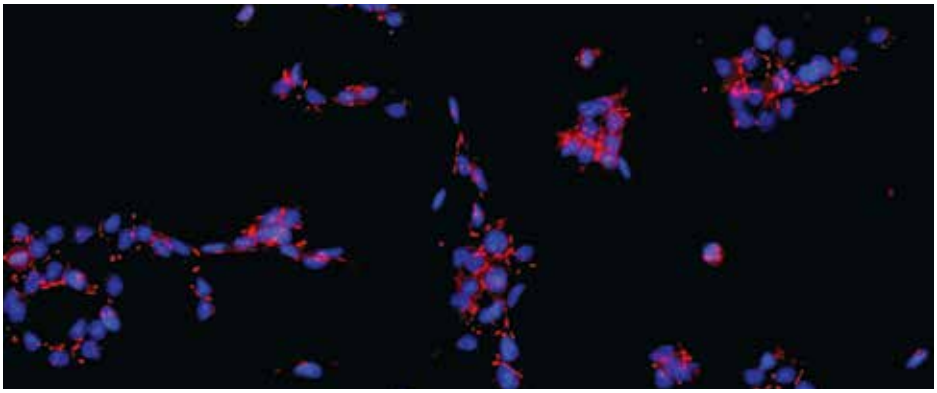
Expression is localized to the extracellular matrix of the placenta. Collagen Type IV is shown in human formalin-fixed paraffin embedded placenta stained with 5 ug/mL Anti-Human Collagen Type IV Purified (cat. no. 14-9871), followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, with DAB visualization. Nuclei are counterstained with hematoxylin.





Basement membrane in FFPE human placenta tissue is visualized with Anti-Human Collagen Type IV eFluor® 660 (red, cat. no. 50-9871) Nuclei are counterstained with DAPI (blue).

JAM3, also known as CD323, is a junctional adhesion molecule expressed on multiple cell types and is involved in anchoring interactions with β integrins. CD323 is involved in neutrophil transmigration during inflammation, angiogenesis, cell polarity, and nerve conduction.

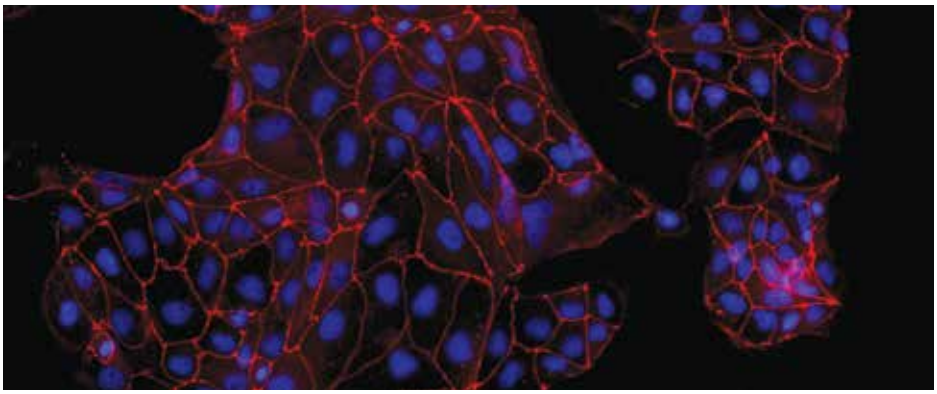


Cell-cell junctions visualized with JAM3

ICC staining of methanol-fixed and permeabilized HEK293 cells using 10 ug/mL Anti-Human CD323 (JAM3) Purified (cat. no. 14-3239) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).

α-catenin is a protein that indirectly mediates interactions with cadherins through β-catenin, α-catenin, and γ-catenins. α-catenin plays a role in cell-cell adhesion through its function in the binding and bundling of actin with the N-terminus of α-catenin binding β-catenin, while the C-terminus binds actin. Monomeric α-catenin acts as a physical linker between the cadherin-β-catenin complex and actin cytoskeleton. It is stabilized by dimeric α-catenin found in the cytoplasm. α-catenin also binds other actin-related proteins, including vinculin, α-actinin, ZO-1, afadin, formin-1, and Rho but is absent in some tumor cell lines and reduced in some human carcinomas.

Zona occludens protein 1, tight-junction protein 1 (ZO-1) is a tight-junction protein and a member of the MAGUK family of tight-junction associated scaffolding proteins. It is an integral member of the tight-junction complex, which forms a barrier to paracellular movement of substances, separating apical and basolateral fluids in relation to the epithelial cell layer. ZO-1 is thought to anchor the actin cytoskeleton to the tight-junction. The α-containing isoform is a scaffold protein localized to clusters associated with epithelial cell junctions. The short isoform is found both in endothelial cells and the epithelial junctions of renal glomeruli and Sertoli cells of the seminiferous tubules. The N-terminal is thought to play a role in signal transduction for assembly of tight-junctions, while the C-terminal of the protein may influence specific properties of tight-junctions. The localization of ZO-1 to the leading edge of migrating cells is thought to play a role in the organization/reorganization of the cytoskeleton during cell movement.



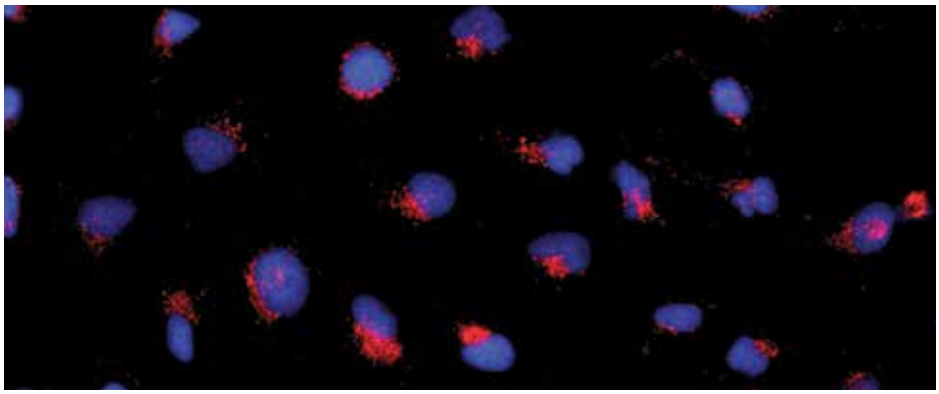
ZO-1 expression localizes to epithelial tight-junctions

Tight-junctions are visualized in formaldehyde-fixed and permeabilized epithelial cells using 10 ug/mL Anti-ZO-1 Purified (cat. no. 41-9776) followed by 10 ug/mL Anti-Rat IgG TRITC (red, cat. no. 26-4826). Nuclei are stained with DAPI (blue).

Lysosome-associated membrane protein family (LAMP) is comprised of five family members: LAMP1, LAMP2, LAMP3, and LAMP5. The LAMP1 protein, also known as CD107a, is a highly glycosylated protein that traverses between lysosomes, endosomes, and the plasma membrane. It is predominantly expressed intracellularly in the lysosomal/endosomal membrane in nearly all cells, in addition to being transiently expressed on the cell surface of degranulating cytolytic T cells, while being upregulated on the surface of activated platelets and some cancer cells. LAMP1 and LAMP 2 appear to be closely related and form the major components of the lysosome membrane, while LAMP3 may play a role in dendritic cell function and adaptive immunity. LAMP5 is localized mainly in the cytoplasmic vesicles of the neuronal cell body, cycling from the endocytotic vesicles to the plasma membrane and accumulating in the endoplasmic reticulum. LAMP5 is expressed in plasmacytoid dendritic cells and appears to be a novel marker for blastic plasmacytoid dendritic cell neoplasia.

Endosomal and lysosomal expression of LAMP1

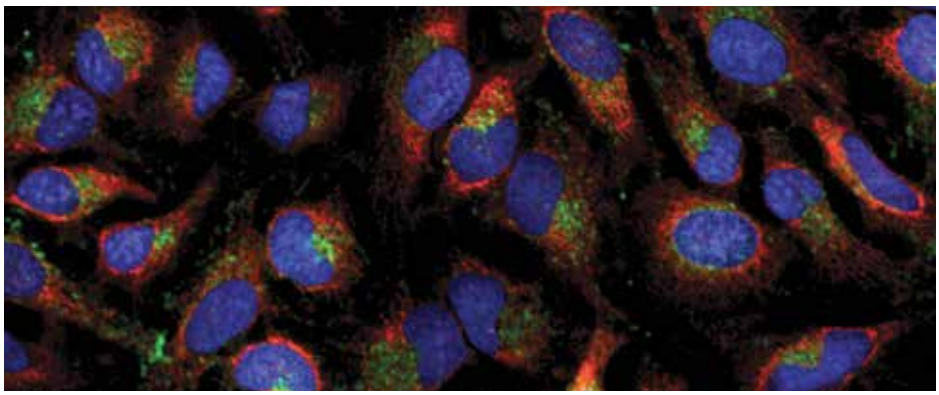
CD107a visualized by ICC staining of methanol-fixed and permeabilized HeLa cells stained with 5 ug/mL Anti-Human CD107a (LAMP-1) Purified (cat. no. 14-1079) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).



COX IV (cytochrome c oxidase), a protein located in the mitochondrial membrane is the terminal enzyme in the mitochondrial respiratory chain. It is localized to the inner membrane of mitochondria and it functions as a cytochrome c oxidase (subunit IV, isoform1), one of the 13 subunits of the COX complex and the terminal enzyme in the mitochondrial respiratory chain. COX IV plays an important role in the transfer of protons across the mitochondrial membrane driving the synthesis of ATP through the ATP synthase.

Detection of mitochondria and lysosomes in human adenocarcinoma cells

COX IV is visualized by staining methanol-fixed HeLa cells using 5 ug/mL Anti-Human COX IV Purified (cat. no. 14-9775) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). CD107a is visualized by staining 10 ug/mL Anti-Human CD107a (LAMP-1) FITC (green). Nuclei are stained with DAPI (blue).

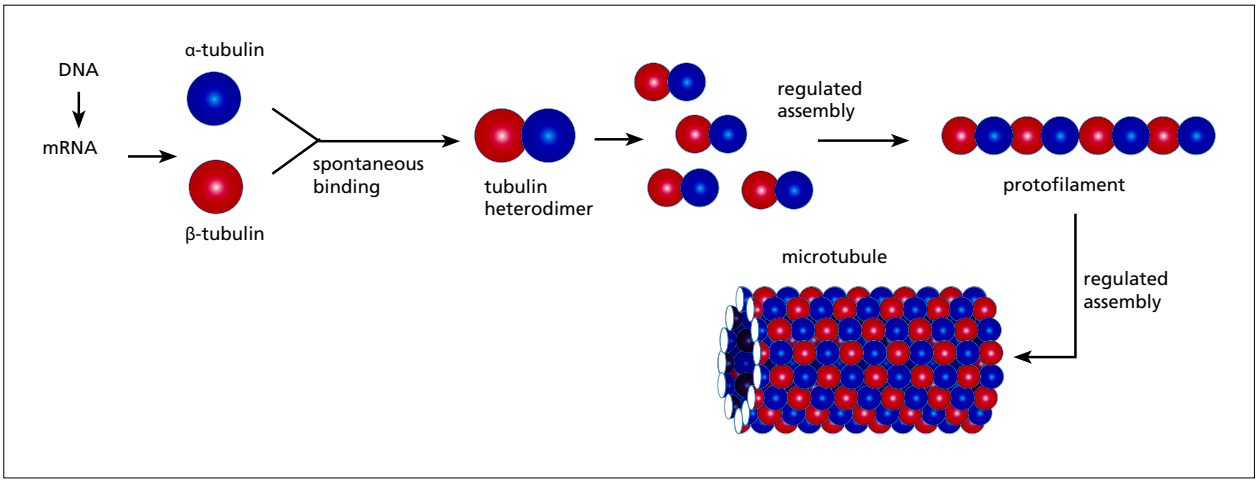


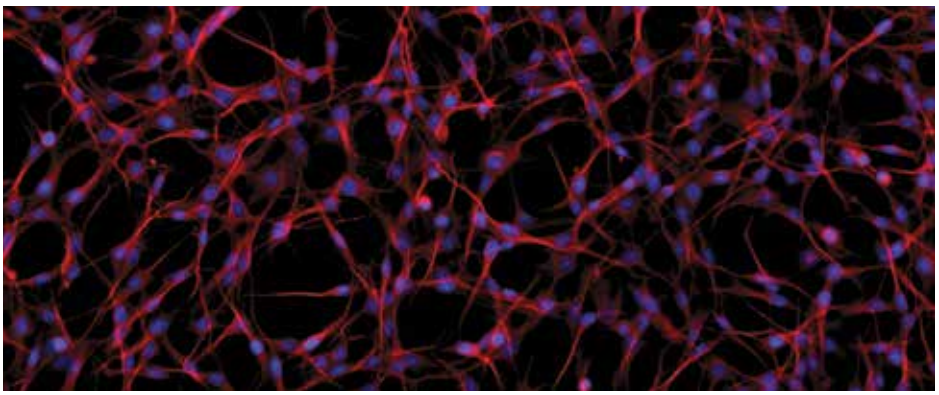
Tubulin to microtubules

The big picture

Tubulin is a heterodimer comprised of an α and β subunit, which when polymerized form protofilaments that become microtubules, involved in cell polarization, migration, intracellular trafficking, and cell division. Microtubule growth and shrinkage is regulated through post-translational modifications, which occur at the C-terminus of the α and β subunit via phosphorylation and acetylation. This results in assembly and polymerization or disassembly and depolymerization of tubulins. The dynamic nature of microtubules is most evident in the mitotic apparatus.

α-tubulin acetylation occurs on lysine 40 by a currently unknown enzyme, however deacetylation via HDAC6 and Sirt2 has been identified *in vivo* and *in vitro*, although function is still under investigation.





α-tubulin filaments localize to the cytoplasm of rat glioma cells

ICC of fixed and permeabilized C6 cells using 1 ug/mL Anti-α-Tubulin eFluor® 615 (red, cat. no. 42-4502). Nuclei are counterstained with DAPI (blue).

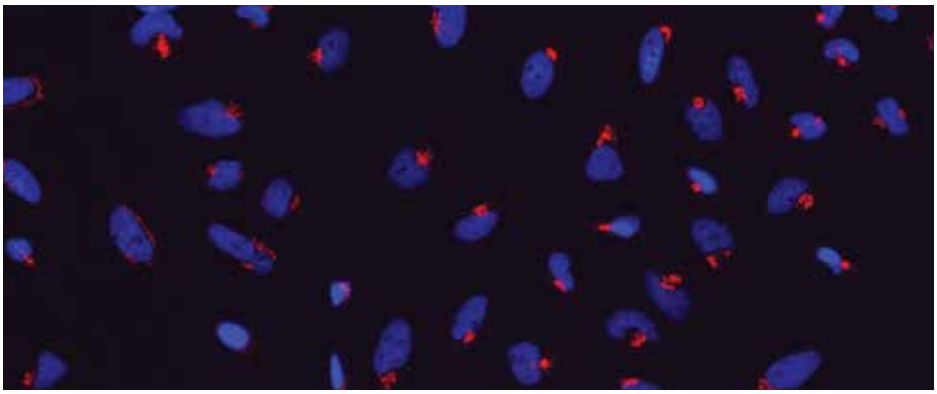
β-tubulin class III (TUBB3) is a member of the extensive tubulin family, comprised of α- and β-tubulins, which heterodimerize to become microtubules of the cytoskeletal network, providing cell shape and structure. β-tubulin class III expression is exclusively found in neuronal cells of adult mammals with potential involvement in neurogenesis, axon guidance, and maintenance. Although expression has been mainly found in neuronal cells, during fetal development, transient expression has been documented in both glial and neuronal precursors localized to the subventricular zone. Identification of β-tubulin class III expression is useful as a structural marker for developing and mature neural cells.

Cell structure: Purified and Biotin formats			
Description	Clone	Purified	Biotin
Anti-Actin (muscle)	HHF35	14-6496	
Anti-Human α-catenin	1G5	14-9120	
Anti-Rat Aminopeptidase P Antibody	JG12	BMS1104	
Anti-Human Bcl-2	Bcl-2/100	14-1028	
Anti-Human/Mouse Bcl-6	GI191E	14-9887	
Anti-Human P-Cadherin	12H6	14-9873	
Anti-Human Cep55	EMRC10-11-55	14-9809	
Anti-Human Collagen Type IV	1042	14-9871	
Anti-Collagen Type X	X53	14-9771	
Anti-Human Cox IV	IB52C31H10	14-9775	
Anti-Human/Mouse Cutaneous Lymphocyte Antigen (CLA)	HECA-452	14-9857	
Anti-CX3CL1 (Fractalkine)	polyclonal	14-7986	
Anti-Mouse/Rat CXCL12α (SDF-1α)	Polyclonal	14-7992	
Anti-Mouse/Rat CXCL12β (SDF-1β)	Polyclonal	14-7991	
Anti-Mouse DLL1 (delta-like 1)	HMD1-5	14-5767	
Anti-Mouse Embigin	G7.43.1	14-5839	
Anti-Human EndoGlyx-1	H572	BMS169	
Anti-Mouse Endomucin	eBioV.7C7	14-5851	
Anti-Rat Pan-Endothelium Marker	HIS52	14-0360	
Anti-ERK1/2	5AD13MA	14-9108	
Anti-Human/Mouse phospho-ERK1/2 (T202/Y204)	MILAN8R	14-9109	
Anti-Mouse ESAM	1G8	14-5852	
Anti-Human Estrogen Receptor β	MC10	14-9336	
Anti-Human FAP	F11-24	BMS168	
Anti-Human Fibronectin	FN-3	14-9869	
Anti-Human FOXA2	3C10	14-4778	
Anti-Human Galectin-3	M3/38	BMS1043	
Anti-Human/Mouse Galectin-3	eBioM3/38	14-5301	
Anti-Glial Fibrillary Acidic Protein (GFAP)	GA5	14-9892	
Anti-Human Glycoprotein VI	HY101	14-9813	
Anti-Grim-19	1A8	14-9937	
Anti-Human/Mouse phospho-H2AX (S139)	CR55T33	14-9865	
Anti-HIF-1α	ESEE122	14-9100	
Anti-Human Lactoferrin	B97	14-6604	
Anti-Mouse Lyve-1	ALY7	14-0443	13-0443
Anti-Human 90K/Mac-2BP	SP-2	BMS146	
Anti-Human MnSOD	MnS-1	BMS122	
Anti-Human/Mouse mTOR	F11	14-2190	
Anti-Smooth Muscle Myosin	SMMS-1	14-6400	
Anti-Myosin Heavy Chain	MF20	14-6503	
Anti-Human Naf1	5C4	14-9903	
Anti-Mouse/Rat Nestin	Rat-401 (4D4)	14-5843	
Anti-Human Neural/Glial Antigen 2 (NG2)	9.2.27	14-6504	
Anti-Human phospho-NF kappa B p65 (S529)	MCFA30	14-9864	
Anti-Human PAR2 (Protease-activated receptor 2)	SAM11	14-9699	
Anti-Human/Mouse Pax5	1H9	14-9918	
Anti-Mouse Plexin-B2	eBio3E7	14-5665	
Anti-Human Podoplanin Antibody	4D5aE5E6	BMS1105	
Anti-Human Podoplanin	NZ-1.3	14-9381	
Anti-Mouse Podoplanin	eBio8.1.1	14-5381	13-5381
Anti-Human Prostate-specific Antigen (PSA)	ER-PR8	14-9110	
Anti-Mouse RAE1δ	RD-41	14-5756	
Anti-Mouse/Rat Receptor Interacting Protein 3 (RIP3)	Polyclonal	14-6048	
Anti-Human SAP (SLAM-Associated Protein)	10C4.2	14-9888	
Anti-Human Synaptophysin	EP10	14-6525	
Anti-Human TARP	eBioTP1 (TP1, a.k.a 1F8)	14-8868	
Anti-Human TCL1	eBio1-21	14-6699	
Anti-Mouse TCR β	H57-597	14-5961	

Cell structure: Purified and Biotin formats			
Description	Clone	Purified	Biotin
Anti-Mouse TLR4/MD-2 Complex	MTS510	14-9924	13-9924
Anti-Human TRAF-1	1F3	14-9791	
Anti-α Tubulin	DM1A	14-4502	13-4502
Anti-Human VAP-1	TK8-14	BMS1033	
Anti-Mouse VEGF Receptor 3	AFL4	14-5988	
Anti-Vimentin	V9	14-9897	
Anti-Vinculin	7F9	14-9777	
Anti-ZO-1	R26.4	14-9776	
Anti-Human CD11a	HI111	14-0119	
Anti-Human CD11a (LFA-1α)	R7.1	BMS102	BMS102BT
Anti-Mouse CD11a	M17/4	14-0111	
Anti-Human CD18 (LFA-1β)	R3.3	BMS103	BMS103BT
Anti-Mouse CD18	M18/2	14-0181	
Anti-Human CD22	eBio4KB128	14-0229	
Anti-Rat CD25	OX39	14-0390	13-0390
Anti-Human CD26	M-A261	BMS143	
Anti-Human CD26	4H3	BMS1023	
Anti-Human CD28	CD28.6	16-0288	
Anti-Human CD28	CD28.2	14-0289	
Anti-Mouse CD28	37.51	14-0281	
Anti-Human CD29 (Integrin β1)	TS2/16	14-0299	
Anti-Mouse CD29	KMI6	14-0292	
Anti-Mouse/Rat CD29 (Integrin β1)	eBioHMb1-1		13-0291
Anti-Human CD31 (PECAM-1)	JC70	14-0318	
Anti-Human CD31 (PECAM-1)	WM-59 (WM59)	14-0319	
Anti-Human CD31 (PECAM-1)	Gi18	BMS137	
Anti-Mouse CD31 (PECAM-1)	390	14-0311	
Anti-Human CD33	WM-53 (WM53)	14-0338	
Anti-Human CD40	5C3	14-0409	
Anti-Mouse CD40	1C10	14-0401	
Anti-Mouse/Rat CD40	HM40-3	14-0402	
Anti-Human CD41a	HIP8	14-0419	
Anti-Human CD42b	HIP1	14-0429	
Anti-Mouse/Rat CD42d	1C2	14-0421	
Anti-Human CD43	eBio84-3C1	14-0439	
Anti-Human/Mouse CD44	IM7	14-0441	
Anti-Human CD44std	SFF-2	BMS113	BMS113BT
Anti-Human CD44std	SFF-304	BMS150	
Anti-Human CD44var (v3)	VFF-327v3	BMS144	
Anti-Human CD44var (v3-v10)	polyclonal	BMS124	
Anti-Human CD44var (v4)	VFF-11	BMS114	BMS114BT
Anti-Human CD44var (v5)	VFF-8	BMS115	
Anti-Human CD44var (v6)	VFF-7	BMS116	
Anti-Human CD44var (v6)	VFF-18	BMS125	
Anti-Mouse CD44var (v6)	9A4	BMS145	
Anti-Mouse CD44var (v4)	10D1	BMS149	
Anti-Human CD44var (v7)	VFF-9	BMS117	BMS117BT
Anti-Human CD44var (v7-v8)	VFF-17	BMS118	BMS118BT
Anti-Human CD45RA	HI100	14-0458	
Anti-Mouse CD45.2	104	14-0454	13-0454
Anti-Human CD45RO	UCHL1	14-0457	
Anti-Mouse CD49b (Integrin α2)	HMa2	14-0491	
Anti-Human CD49d (Integrin α4)	9F10	14-0499	
Anti-Mouse CD49d (Integrin α4)	R1-2	14-0492	
Anti-Human CD49e (Integrin α5)	eBioSAM-1 (SAM1)	14-0496	
Anti-Human/Mouse CD49f	eBioGoH3	14-0495	13-0495
Anti-Human CD50 (ICAM-3)	CBR-IC3/1	BMS111	BMS111BT
Anti-Human CD51/CD61	23C6	14-0519	

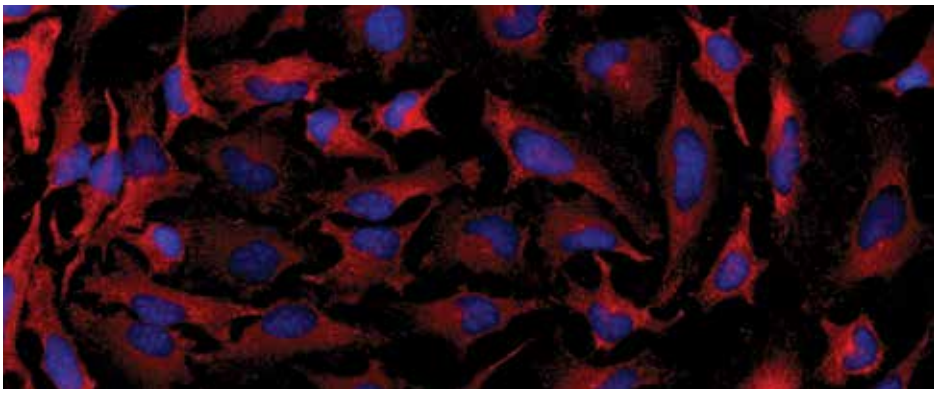
Cell structure: Purified and Biotin formats			
Description	Clone	Purified	Biotin
Anti-Mouse CD54 (ICAM-1)	YN1/1.7.4	14-0541	
Anti-Mouse CD54 (ICAM-1)	eBioKAT-1 (KAT1)	14-0542	
Anti-Human CD54 (ICAM-1)	HA58	14-0549	
Anti-Human CD54 (ICAM-1)	RR1/1	BMS108	
Anti-Human CD61 (Integrin β 3)	VI-PL2	14-0619	
Anti-Mouse/Rat CD61 (Integrin β 3)	2C9.G3	14-0611	
Anti-Human CD62E (E-Selectin)	P2H3	14-0627	
Anti-Human CD62E (E-Selectin)	CL-37	BMS1014	
Anti-Human CD62E (E-Selectin)	CL2	BMS110	BMS110BT
Anti-Human CD62L (L-Selectin)	DREG.55	BMS121	BMS121BT
Anti-Human CD62L (L-Selectin)	DREG-56 (DREG56)	14-0629	
Anti-Human CD62L (L-Selectin)	DREG.200	BMS1015	
Anti-Mouse CD62L (L-Selectin)	MEL-14	14-0621	
Anti-Human CD62P (P-Selectin)	AK-4	14-0628	
Anti-Human CD66e (CEA)	CB30	14-0669	
Anti-Mouse CD66a (CEACAM1)	CC1	14-0661	
Anti-Mouse CD73	eBioTY/11.8	14-0731	
Anti-Human CD93	R139	14-0939	
Anti-Mouse CD100 (SEMA4D)	BMA12 (BMA-12)	14-1001	
Anti-Human CD102 (ICAM-2)	CBRIC2/2	14-1029	
Anti-Human CD102 (ICAM-2)	CBR-IC2/2	BMS109	
Anti-Mouse CD102 (ICAM-2)	3C4 (mIC2/4)	14-1021	
Anti-Human CD103 (Integrin α-E)	B-Ly7	14-1038	
Anti-Mouse CD103 (Integrin α-E)	2E7	14-1031	
Anti-Human CD104 (Integrin β-4)	439-9B	14-1049	13-1049
Anti-Mouse CD105 (Endoglin)	MJ7/18	14-1051	
Anti-Human CD106 (VCAM-1)	STA	14-1069	
Anti-Human CD106 (VCAM-1)	B-N8	BMS141	BMS141BT
Anti-Mouse CD106 (VCAM-1)	429	14-1061	
Anti-Rat CD106 (VCAM-1)	eBioMR106	14-1060	
Anti-Human CD107a (LAMP-1)	eBioH4A3	14-1079	
Anti-Mouse CD107a (LAMP-1)	eBio1D4B	14-1071	
Anti-Mouse CD107b (LAMP-2)	eBioABL-93	14-1072	
Anti-Mouse CD107b (Mac-3)	M3/84	14-5989	
Anti-Human CD115 (c-fms)	12-3A3-1B10	14-1159	
Anti-Mouse CD115 (c-fms)	AFS98	14-1152	
Anti-Human CD124	X2/45-12	14-1249	
Anti-Human CD127	eBioRDR5	14-1278	
Anti-Mouse CD127	A7R34	14-1271	
Anti-Mouse CD130	KGP130	14-1302	
Anti-Human CD138 (Syndecan-1)	DL-101	14-1389	
Anti-Mouse CD140a (PDGF Receptor a)	APA5	14-1401	
Anti-Mouse CD140b (PDGF Receptor b)	APB5	14-1402	
Anti-Human CD144 (VE-Cadherin)	16B1	14-1449	13-1449
Anti-Human CD144 (VE-Cadherin)	polyclonal	BMS158	BMS158BT
Anti-Mouse CD144 (VE-Cadherin)	eBioBV13	14-1441	13-1441
Anti-Mouse CD144 (VE-Cadherin)	eBioBV14	14-1442	13-1449
Anti-CD146	P1H12	14-1469	
Anti-Human CD147	8D12	14-1472	
Anti-Mouse CD147	RL73	14-1471	
Anti-Human CD162 (PSGL-1)	PL-1	BMS164	
Anti-Human CD163	eBioGHI/61	14-1639	
Anti-Mouse CD166 (ALCAM)	eBioALC48	14-1661	
Anti-Human CD171	eBio5G3	14-1719	13-1719
Anti-Human CD180 (RP105)	MHR73-11	14-1809	
Anti-Human CD181 (CXCR1)	eBio8F1-1-4	14-1819	
Anti-Human CD182 (CXCR2)	eBio5E8-C7-F10	14-1829	
Anti-Mouse CD184 (CXCR4)	2B11	14-9991	

Cell structure: Purified and Biotin formats			
Description	Clone	Purified	Biotin
Anti-Human CD195 (CCR5)	eBioT21/8	14-1957	13-1957
Anti-Human CD197 (CCR7)	3D12	14-1979	
Anti-Human CD218a (IL-18 Receptor α)	H44	14-7183	
Anti-Human CD227 (Mucin 1)	SM3	14-9893	
Anti-Human CD266 (TWEAK Receptor)	ITEM-1	14-9019	
Anti-Human/Mouse CD266 (TWEAK Receptor)	ITEM-4	14-9018	
Anti-Human CD270 (HVEM)	eBioHVEM-122	14-5969	
Anti-Human CD281 (TLR1)	GD2.F4	14-9911	
Anti-Human CD282 (TLR2)	TL2.1	14-9922	
Anti-Human CD282 (TLR2)	TL2.3	14-9029	
Anti-Human/Mouse CD282 (TLR2)	T2.5	14-9024	
Anti-Mouse CD282 (TLR2)	6C2	14-9021	13-9021
Anti-Mouse CD282 (TLR2)	mT2.7	14-9022	
Anti-Human CD283 (TLR3)	TLR3.7	14-9039	
Anti-Human CD284 (TLR4)	HTA125	14-9917	
Anti-Human CD286 (TLR6)	hPer6	14-9069	
Anti-Human CD289 (TLR9)	eB72-1665	14-9099	
Anti-CD324 (E-Cadherin)	DECMA-1	14-3249	13-3249
Anti-Human CD326 (EpCAM)	1B7	14-9326	13-9326
Anti-Human CD326 (EpCAM)	VU-1D9	BMS171	
Anti-Mouse CD326 (EpCAM)	G8.8	14-5791	



Golgin-97 localizes to the golgi apparatus

Immunocytochemistry of fixed and permeabilized HeLa cells stained with 1 ug/mL Anti-Golgin-97 Purified (cat. no. 14-9767) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).

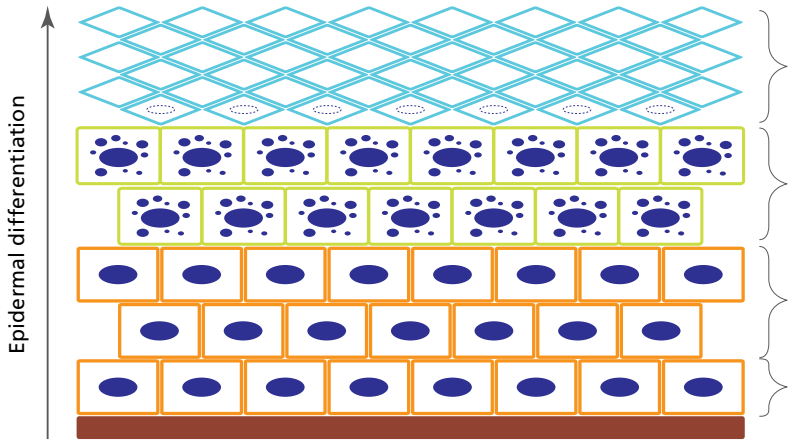


Detection of Dynein intermediate chain along microtubules

Dynein is visualized by staining methanol-fixed and permeabilized HeLa cells using 1 ug/mL Anti-Dynein Purified (cat. no. 14-9773) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).

Cell structure: eFluor® and Organic Dye formats							
Description	Clone	FITC	Alexa Fluor® 488	eFluor® 520	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Actin (muscle)	HHF35		53-6496				
Anti-Human α-Catenin	1G5				41-9120		
Anti-Human Collagen Type IV	1042						50-9871
Anti-Collagen Type X	X53				41-9771		
Anti-Human Cox IV	IB52C31H10		53-9775		41-9775		
Anti-Human/Mouse Cutaneous Lymphocyte Antigen (CLA)	HECA-452						50-9857
Anti-Human/Mouse High Endothelial Venule Marker	MECA-79		53-6036				
Anti-Human Fibronectin	FN-3		53-9869				50-9871
Anti-Glial Fibrillary Acidic Protein (GFAP)	GA5		53-9892			42-9892	50-9892
Anti-Grim-19	1A8	11-9937					
Anti-Human/Mouse phospho-H2AX (S139)	CR55T33				41-9865		50-9865
Anti-Mouse Lyve-1	ALY7		53-0443		41-0443	42-0443	50-0443
Anti-Smooth Muscle Myosin	SMMS-1						50-6400
Anti-Myosin Heavy Chain	MF20		53-6503				50-6503
Anti-Human Neural/Glial Antigen 2 (NG2)	9.2.27		53-6504				
Anti-Human phospho-NF κ B p65 (S529)	MCFA30				41-9865		
Anti-Human PAR2 (Protease-activated receptor 2)	SAM11						50-9699
Phalloidin				59-6559	41-6559		50-6559
Anti-Human Runx3	R3-5G4						50-9817
Anti-Human Synaptophysin	EP10					42-6525	50-6525
Anti-Human TRAF-1	1F3				41-9791		
Anti-α Tubulin	DM1A		53-4502				
Anti-β Tubulin Class III	2G10-TB3				41-4510		50-4510
Anti-Vimentin	V9	11-9897			41-9897	42-9897	50-9897
Anti-Vinculin	7F9		53-9777				
Anti-ZO-1	R26.4				41-9776		
Anti-Mouse CD31 (PECAM-1)	390	11-0311					
Anti-Mouse CD102 (ICAM-2)	3C4 (mIC2/4)		53-1021				
Anti-Human CD104 (Integrin β-4)	439-9B						50-1049
Anti-Human CD144 (VE-Cadherin)	16B1		53-1449				
Anti-Mouse CD144 (VE-Cadherin)	eBioBV13		53-1441				
Anti-Human CD227 (Mucin 1)	SM3		53-9893			42-9893	
Anti-Human CD323 (JAM3)	SHM33				41-3239		
Anti-CD324 (E-Cadherin)	DECMA-1		53-3249				

Tissue structure



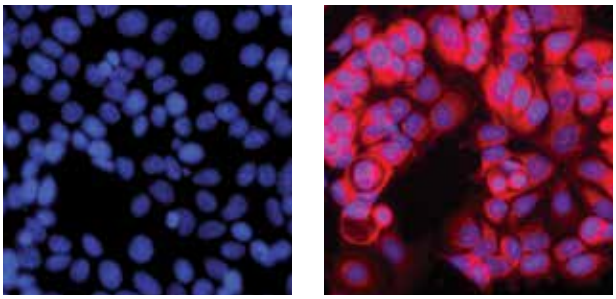
Cornified layer (stratum corneum)
Loricrin, Involucrin, Trichohyalin, S100 proteins, Small proline-rich proteins

Granular layer
Cytokeratin 1, 2, and 10, Profilaggrin, Transglutaminase 3

Spinous layer
Transglutaminase 1 and 5

Spinous layer
Cytokeratin 5 and 14

Basic and acidic cytokeratin pairing with tissue localization		
Basic Group II	Tissue expressed	Acidic Group I
Cytokeratin 1	Keratinocytes	Cytokeratin 10
Cytokeratin 2	Palmoplantar keratinocytes	Cytokeratin 9
Cytokeratin 3	Corneal epithelium	Cytokeratin 12
Cytokeratin 4	Non-keratinized squamous epithelium, including transitional epithelium. CK4 includes cornea	Cytokeratin 13
Cytokeratin 5	Keratinocytes of stratified squamous epithelia are expressed in basal and myoepithelial cells of complex and glandular epithelial tissues	Cytokeratin 14
Cytokeratin 6	Epidermis, nail epithelia, and non-keratinizing stratified squamous epithelia, but are also expressed in ductal luminal	Cytokeratin 16
	cells and in secretory cells of human eccrine sweat glands	
Cytokeratin 7	Simple epithelia, mesothelium, urothelium, and pseudostratified epithelium	Cytokeratin 19
Cytokeratin 8	Epithelial and carcinoma cells	Cytokeratin 18
	Basal layer of epidermis, esophagus, and exocervix	Cytokeratin 15
	Basal/myoepithelial cell keratin characteristically induced after skin injury. Expressed in outer root sheath and medulla region of hair follicle	Cytokeratin 17
	Gastrointestinal epithelia, the urothelium, and in Merkel (neuroendocrine) cells of the skin	Cytokeratin 20



Cytokeratin 7 expression localizes to the cytoskeleton of human epithelial cells

Immunocytochemistry of fixed and permeabilized MCF-7 cells using 10 ug/mL Mouse IgG2b K Isotype Control eFluor® 615 (cat. no. 42-4732, left) or 10 ug/mL Anti-Human Cytokeratin 7 eFluor® 615 (cat. no. 42-9005, right). Nuclei are counterstained with DAPI (blue).

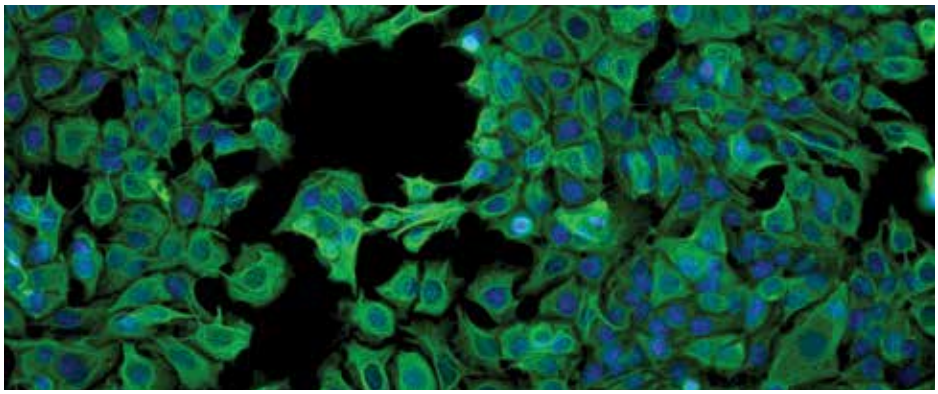
Cytokeratins (CKs) or keratins are intermediate filaments (IFs) predominantly expressed in epithelial cells and some non-epithelial cells. Cytokeratins form the intracellular cytoskeletal network that maintains the integrity and stability of cells and tissues. These keratin proteins can be categorized into two families: acidic group, containing CK9 to CK20, and basic, group comprised of CK1 to CK8. Most cytokeratins in group I pair with a cytokeratin in group II to form a heteropolymer, sharing a common structure that consists of a central coiled-coil α-helical rod domain, that is flanked by non-helical head and tail domains.

Pairing of a basic and acidic keratin is specific for different epithelial cells, allowing for the identification of epithelial cell origin, which is important in classifying both tumor cells and metastases. The use of pan-cytokeratin antibodies is useful for initial identification or establishment of the epithelial-origin of a neoplasm. AE1/AE3 clone detects CK1-8, 10, 14-16, and 19, but does not detect CK17 or CK18.

Because cytokeratins are highly expressed in an organized and specific pattern within different types of epithelial cells, they are ideal markers for the identification of epithelial malignancies. Aside from the role keratins play in maintaining cell structure, some keratins have been found to serve functional roles in regulating membrane trafficking and epithelial polarity. Recent studies have shown that carcinoma and apoptotic cells can release keratin fragments from the cell into the circulation, making them suitable biomarkers for measuring tumor load. Some cytokeratin motifs act as substrates for caspase degradation during intermediate apoptosis events. Studies have demonstrated the involvement of cytokeratin 8 in protection against apoptosis, stress, or injury in addition to regulating the cell cycle. This keratin is frequently co-expressed with cytokeratin 18, a type I (acidic) keratin as a heterodimer. Although detected primarily in the cytoplasm of normal healthy cells, cytokeratin 8 has been found to localize to the plasma membrane in some tumor cells. Cytokeratin 8 is phosphorylated on serine 73 in dividing cells. Cytokeratin 19 (CK19), a 44-kDa type I (acidic) intermediate filament protein lacks the non-α-helical tail domain present in other keratins and is expressed in a wide variety of simple and stratified epithelial tissue. Cytokeratin 19 expression can be induced by vitamin A, SV40 transformation, and cancer. A soluble form of cytokeratin 19 generated by caspase 3 cleavage has also been found to be secreted by cancer cells, possibly indicating tumor metastasis. Cytokeratin 19 often exists as a heterodimer with cytokeratin 7, a type II keratin. Cytokeratin staining can be observed in most carcinomas, tumors, and epithelial organs.

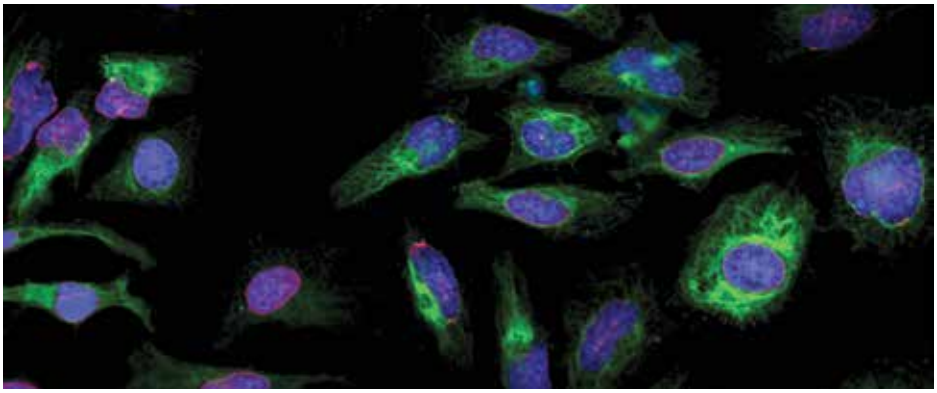
Cytokeratin 18 staining highlights cytoplasmic filaments in mcf-7 cells

Filaments are visualized in methanol-fixed and permeabilized MCF7 human breast adenocarcinoma cells using 5 ug/mL Anti-Human Cytokeratin 18 Alexa Fluor® 488 (green, cat. no. 53-9815). Nuclei are counterstained with DAPI (blue).



Tissue structure antibodies listed by format							
Description	Clone	Purified	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Acidic Cytokeratin	AE1	14-9001					
Anti-Basic Cytokeratin	AE3	14-9000					
Anti-Pan Cytokeratin (AE1/AE3)	AE1/AE3			53-9003	41-9003	42-9003	50-9003
Anti-Human Cytokeratin 6	LL020	14-9788					
Anti-Human Cytokeratin 7	LP5K	14-9005				42-9005	
Anti-Human Cytokeratin 8	LP3K	14-9938	11-9938				
Anti-Human Cytokeratin 18	LDK18	14-9815		53-9815	41-9815		
Anti-Human Cytokeratin 19	BA17	14-9898	11-9898	53-9898		42-9898	

Lamins are a class of intermediate filament proteins forming a two-dimensional matrix of the nuclear lamina. Lamin proteins are highly conserved and function to assemble and disassemble the lamina matrix following phosphorylation. Lamin proteins are likely to be involved in stabilizing the nucleus and chromatin and may play a role in gene regulation. Vertebrate lamins consist of two types, A and B, which can be alternatively spliced to generate multiple isoforms. Depolymerization of the nuclear lamins leads to disintegration of the nuclear envelope.

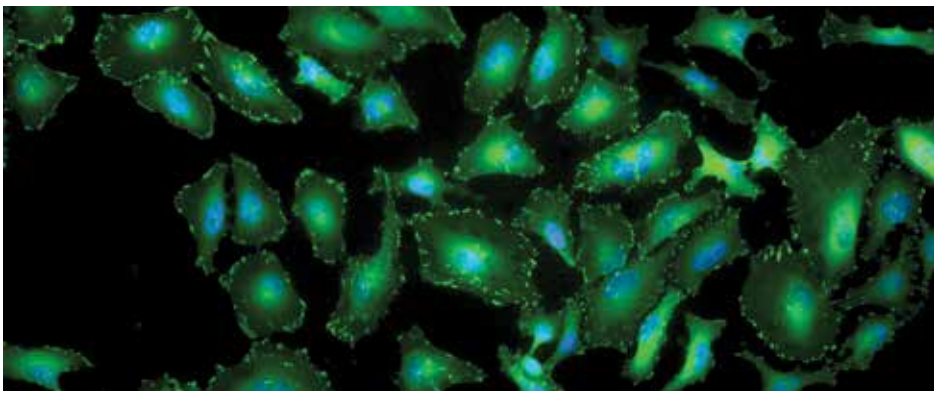


Lamin and cytokeratin staining of intermediate filament proteins in human adenocarcinoma cells

Lamins comprise the nuclear lamina, a filamentous network underneath the inner nuclear envelope of the cell. Lamin A/C is visualized by ICC staining of methanol-fixed HeLa cells using 10 ug/mL Anti-Lamin A/C Purified followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010) . Cytokeratins are visualized using 1 ug/mL Anti-Pan-Cytokeratin (AE1/AE3) Alexa Fluor® 488 (green, cat. no. 53-9003). Nuclei are stained with DAPI (blue).

Grim-19 is primarily localized to mitochondria as an essential component of complex I of the electron transport chain. Grim-19 expression can be induced by interferon-β and all-trans-retinoic acid in tumor cell lines, leading to cell death. Finally, Grim-19 has been shown to bind the transactivation domain of STAT3 and inhibit its transcriptional activity. The molecular mechanisms of how Grim-19 regulates STAT3 within the cell remains under active investigation.

Vinculin and its alternatively spliced isoform, metavinculin, are cytoskeletal proteins associated with cell-cell and cell-matrix junctions. Vinculin is involved in the anchoring of F-actin to the membrane and the regulation of E-Cadherin expression. Vinculin binds to talin, paxillin, and α-actinin. Dysregulation of vinculin alters cell adhesion, migration, and growth, which can promote cancer invasion and metastasis.



Vinculin expression localizes to focal adhesion plaques

Expression is detected in formaldehyde-fixed and permeabilized HeLa cells using 20 ug/mL Anti-Vinculin Alexa Fluor® 488 (green, cat. no. 53-9777). Staining is localized to the cytoskeleton and F-actin anchoring sites throughout the cell. Nuclei are stained with DAPI (blue).

4

Cancer

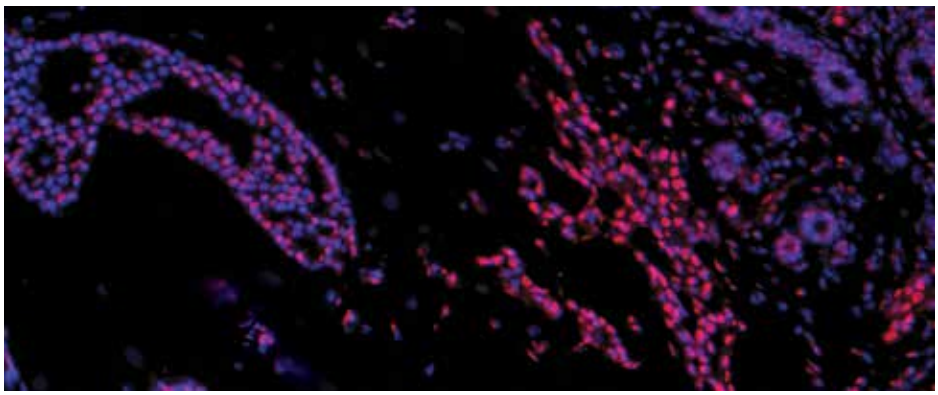
Half of all men and one-third of women will be affected by some form of cancer. This debilitating, often deadly, disease may begin as breast cancer, which often metastasizes to the brain, while prostate cancer is known to travel to the bone. Genes are localized along chromosomes, which are then transcribed and translated within the cell nucleus and cytoplasm respectively. Understanding expression patterns and levels leading to the creation of a particular phenotype is crucial to the advancement of research. Expressed proteins have many functions ranging from cell signaling to maintenance of cytoarchitecture, however, genomic sequences are prone to corruption by various mechanisms and factors, resulting in mutations and downstream dysregulation of protein levels, and ultimately, function. Cancer is thought to occur through both accumulation of these mutations as well as through changes in post-translation modifications (PTM) and signal transduction. Cancer cells stimulate their own growth-resisting inhibitory signals that might otherwise halt proliferation and initiate apoptosis. Continued growth and division of cancer cells share many characteristics of stem cells, which allow the primary tumor to multiply indefinitely, invade local tissue, and metastasize to blood and lymphatic vessels, which provide access to nutrients along with entry to distant sites.

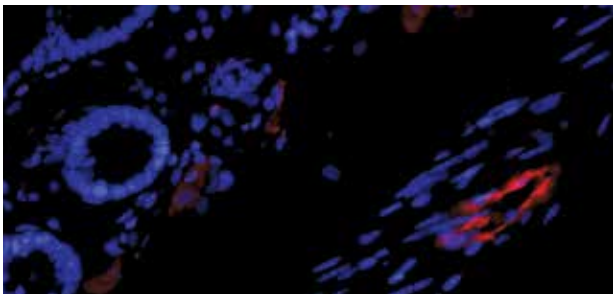
The field of Cancer Biology has seen an increased focus on the interdependence of immune cells, in addition to the development and progression of tumors. The association and densities of cytotoxic T cells (CTL), regulatory T cells (Tregs), and Th17 cells within the tumor and its microenvironment are studied as potential indicators of tumor stage-progression. It is also important to evaluate development of basic cell and tissue structural elements, for example during angiogenesis, as well as activation and proliferation using critical markers such as Ki-67. eBioscience actively develops quality IHC and ICC tools to support this key research area.

Cyclins regulate the cell cycle by binding and activating cyclin-dependent kinases (CDK). Cyclin E regulates the transition from G_1 to S phase by binding and activating CDK2. The Cyclin E/CDK2 complex is responsible for the phosphorylation of downstream proteins that facilitate the G_1 to S phase transition. Overexpression of Cyclin E is found in several types of cancer including breast, colon, bladder, lung, and skin. In breast cancer, Cyclin E overexpression is associated with increased stage and grade in addition to poor prognosis.

Immunofluorescent detection of Cyclin E

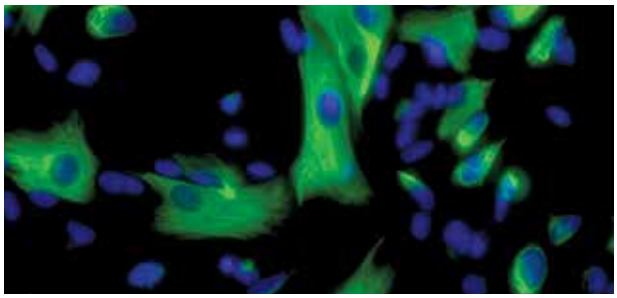
Cyclin E expression is up-regulated in ductal epithelial cells of a grade 2 infiltrating ductal carcinoma. Cyclin E is detected in a formalin-fixed paraffin embedded human tissue section using 5 ug/mL Anti-Human Cyclin E eFluor® 660 (red, cat. no. 50-9714). Nuclei are stained with DAPI (blue). Colocalization appears pink.





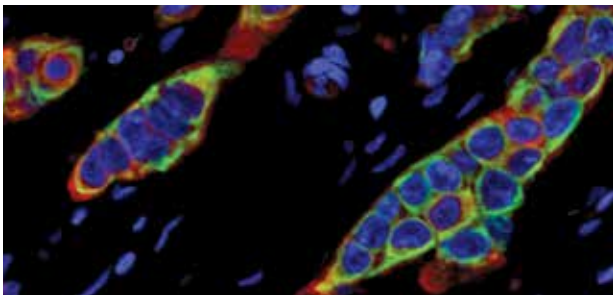
LYVE-1 eFluor® 660

LYVE-1, a receptor for PDGF-B and VEGF-A, visualized by immunohistochemical staining of mouse intestine using 10 ug/mL Anti-Mouse LYVE-1 eFluor® 660 (cat. no. 50-0443) and DAPI (blue).



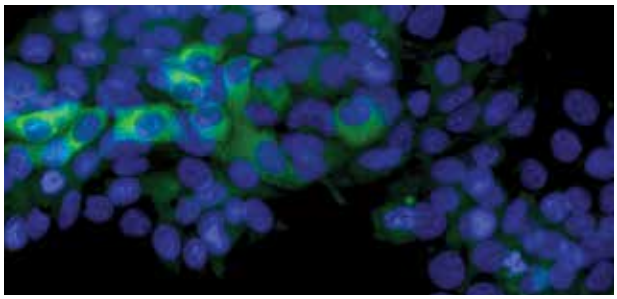
Vimentin FITC

Vimentin, an intermediate filament expressed in subsets of progenitor cells, visualized by immunocytochemical staining of fixed, permeabilized SK-N-SH human neuroblastoma cells using 1 ug/mL Anti-Vimentin FITC (cat. no. 11-9897). Nuclei are counterstained with DAPI (blue).



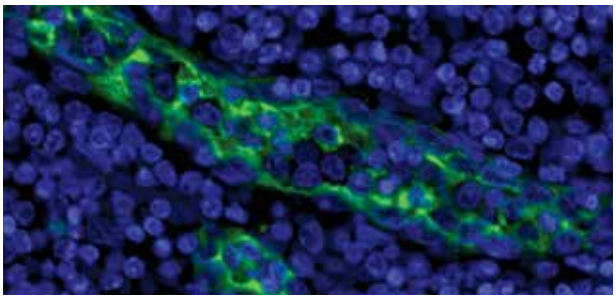
Heat Shock Protein 27 eFluor® 660 and Pan-Cytokeratin (AE1/AE3) Alexa Fluor® 488

10 ug/mL Anti-Human Heat Shock Protein 27 eFluor® 660 (red, cat. no. 50-9112), known to be upregulated in breast cancer, is co-expressed with the cytoskeletal marker 1 ug/mL Anti-Pan-Cytokeratin Alexa Fluor® 488 (green, cat. no. 53-9003) in the cytoplasm of ductal epithelial cells in formalin-fixed paraffin embedded human infiltrating ductal carcinoma. Nuclei are counterstained with DAPI (blue).



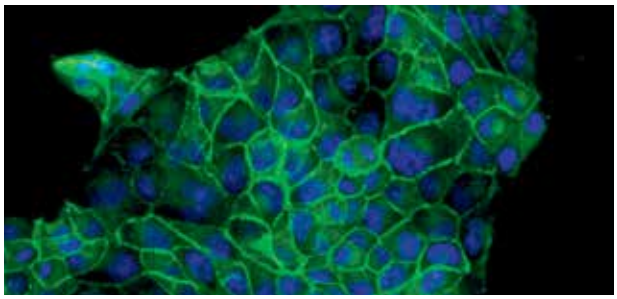
Chorionic Gonadotropin Alexa Fluor® 488

Chorionic Gonadotropin (hCG), an important regulator of angiogenesis and activator of the PI3K/mTOR pathway, visualized in fixed, permeabilized BeWo cells stained using 20 ug/mL Anti-Human Chorionic Gonadotropin (hCG) Alexa Fluor® 488 (cat. no. 53-6508). Nuclei are counterstained with DAPI (blue).



High Endothelial Venule Marker Alexa Fluor® 488

20 ug/mL Anti-Human/Mouse High Endothelial Venule Marker Alexa Fluor® 488 (cat. no. 53-6036). Meca79 detects a glycoprotein expressed on the luminal surface and in the cytoplasm of high endothelial venules (HEV), localized sites of lymphocyte infiltration and adhesion. Expression is visualized in FFPE human tonsil and nuclei are stained with DAPI (blue).



E-Cadherin (CD324) Alexa Fluor® 488

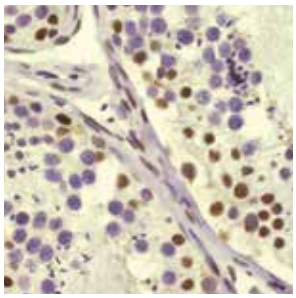
E-Cadherin, an adhesion molecule that can display multiple functions in stem cell and cancer/EMT biology, is visualized in fixed, permeabilized MDCK cells stained using 20 ug/mL Anti-E-Cadherin Alexa Fluor® 488 (cat. no. 53-3249). Nuclei are counterstained with DAPI (blue).

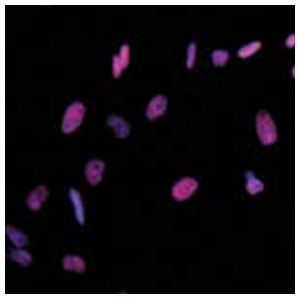
Brachyury, a 47 kDa protein encoded by the T gene, functions as a nuclear transcription factor. This transcription factor binds to specific DNA regions near the palindromic sequence TCACACCT (T-site) through a region in its N-terminus, called the T-box. Brachyury functions during embryogenesis to regulate midline development by establishing the anterior/posterior axis through the regulation of genes involved in mesoderm formation and differentiation. Brachyury is the primary mesoderm marker used to distinguish differentiation of embryonic stem cells into a mesenchymal stem cell lineage. Variations in human T genes are associated with neural tube defects and chordomas. Expression of brachyury is also increased in advanced-stage lung tumors and tumor cell lines.

Expression and localization of Brachyury

Brachury expression is localized to germinal epithelium in human formalin-fixed paraffin embedded testes tissue (left). Expression of Brachyury is detected using 5 ug/mL Anti-Human Brachyury Purified (cat. no. 14-9770), followed by Anti-Mouse IgG Biotin and Streptavidin HRP with DAB visualization. Nuclei are counterstained with hematoxylin.

Brachyury expression is localized to the nucleus of formaldehyde-fixed and permeabilized human cervical adenocarcinoma cells using 5 ug/mL Anti-Human Brachyury Purified (cat. no. 14-9770) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).



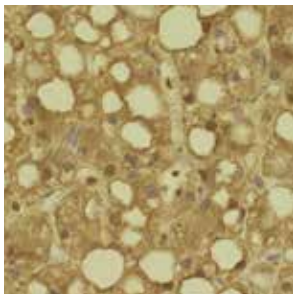


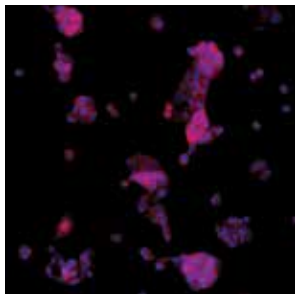
Arginase-1 is a 35 kDa enzyme that helps eliminate nitrogen by converting L-arginine to urea and L-ornithine conversion to polyamine. Arginase converts L-arginine into L-ornithine and urea, as a final step of the urea cycle. Polyamines are important for cell proliferation and removal of toxins that arise from protein degradation. Expression is found in the liver, neutrophils, macrophages (M2 or alternatively activated macrophages), as well as myeloid-derived suppressor cells (MDSC) and neural stem cells. Unlike hepatocytes, expression of Arginase-1 is tightly controlled in macrophages. Th2 cytokines, such as IL-4 and IL-13, have been shown to upregulate Arginase-1 expression, which in turn promotes inflammation and slows progression of Th2-mediated responses. Changes in Arginase-1 levels have been associated with cancer.

Arginase-1 localization in the cytoplasm

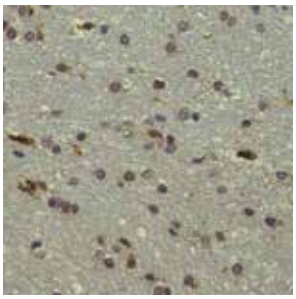
Expression of arginase-1 localizes to the cytoplasm of hepatocytes. Arginase is visualized in human formalin-fixed paraffin embedded tissue sections using 5 ug/mL Anti-Human Arginase-1 Purified (cat. no. 53-9779), followed by Anti-Mouse IgG Biotin, Streptavidin HRP, with DAB visualization. Nuclei are counterstained with hematoxylin.

Arginase expression is localized to the cytoplasm of formaldehyde-fixed, permeabilized HepG2 human liver carcinoma cells using 20 ug/mL Anti-Human Arginase-1 Purified, followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue).





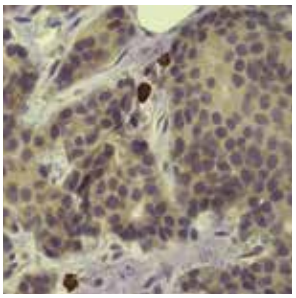
TAL-1 protein is encoded by the T-acute leukemia (TAL-1) or stem cell leukemia (SCL) gene and functions as a 42 kDa basic helix loop, helix transcription factor involved in mediating cell growth and differentiation. Rearrangements of the TAL-1 gene are common in human T-cell acute leukemias (T-ALLs) and result in aberrant transcription in leukemic cells. TAL-1 expression in leukemic cells is localized to the nucleus, although both nuclear and cytoplasmic expression has been identified in normal cells, such as erythroid precursors, megakaryocytes, endothelial cells, and macrophages scattered throughout tissues, such as the tonsil, cerebellum, kidney, lung, and spleen. TAL-1 is known to regulate both embryonic and adult hematopoiesis and more recently was found to regulate proliferation and differentiation of common myeloid precursors to monocytes and, ultimately, macrophages within tissue.



Colorimetric detection of TAL-1-positive microglia

Microglial cells scattered throughout the cerebellar white matter in a formalin-fixed paraffin embedded human tissue section are detected using 20 ug/mL Anti-Human TAL-1 Purified (cat. no. 14-9101), followed by Anti-Mouse IgG Biotin, Streptavidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

Globo H is a hexasaccharide formed from the pentasaccharide precursor, SSEA3 (stage-specific embryonic antigen 3). It is a member of antigenic carbohydrates expressed on the surface of cancer cells as glycolipids and possibly glycoproteins. Globo H is overexpressed on many carcinomas, such as breast, colon, ovarian, gastric, pancreatic, lung, and prostate. Expression is observed in more than half of all ductal, lobular, and tubular breast carcinomas, but not in non-epithelial breast tumors. Globo H is also expressed on cancer stem cells and human embryonic stem cells, although expression decreases when cells are differentiated toward both neural progenitor cells and to a lesser extent in endodermal lineage cells. Expression in normal tissues is limited to cells forming secretory borders. Globo H is considered a target for immunotherapy of many epithelial cancers.



Globo H overexpression in breast cancer

Globo H overexpression is detected in ductal epithelial cells of a grade 2 infiltrating ductal carcinoma. Globo H is detected in a formalin-fixed paraffin embedded human tissue section using 5 ug/mL Anti-Human Globo H Purified (cat. no. 14-9700), followed by Anti-Mouse IgG Biotin, Streptavidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

HER family dysregulated in cancer

Receptor tyrosine kinases involved in multiple cell processes

The Epidermal growth factor receptor (EGFR) family is comprised of four structurally similar receptor tyrosine kinases: HER1 (EGFR), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) which are known to affect a variety of cell processes including proliferation, differentiation, migration, survival, and apoptosis and are often dysregulation in cancer.

HER1 (EGFR) is a cell-surface protein that binds to the epidermal growth factor, leading to cell proliferation as a result of dimerization and tyrosine autophosphorylation. Overexpression of EGFR is one of the earliest and most consistent abnormalities in bronchial epithelium of high-risk smokers and is pronounced in virtually all squamous carcinomas in addition to more than 65% of large cell and adenocarcinomas, but is not expressed *in situ* in most small-cell lung carcinomas.

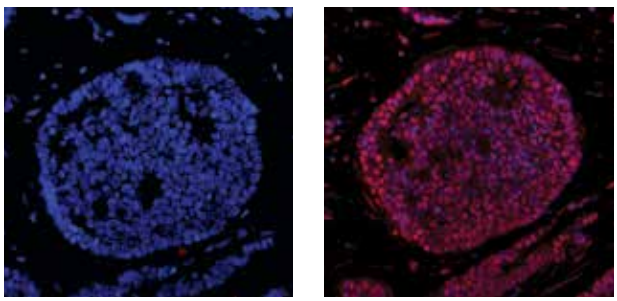
HER2 (ErbB2) is useful in identifying cancer cells with increased aggressiveness. Soluble p97-115 HER-2 (the soluble circulating fragment of p185 HER-2) protein levels in serum can be used as a diagnostic tool for monitoring the extent of tumor spread, postoperative relapse, or metastatic risk for different cancers. Thirty-two percent of ovarian carcinomas overexpress the HER-2 oncoprotein. Survival of those patients is significantly worse compared with cases of normal HER-2 protein expression. A correlation between HER-2 expression and clinical outcome has also been demonstrated for head, neck, salivary gland, and placental carcinomas.

HER3 (ErbB3) is expressed on normal human tissues, particularly on neurons and cells of the gastrointestinal tract. c-ErbB3 is overexpressed in a variety of cancers, including breast, ovary, and gastrointestinal carcinomas. Upon binding its ligand heregulin β-1, c-ErbB3 is activated by heterodimerization with ErbB2 or EGFR1. c-ErbB3 mediates tumor cell invasion, intravasation, and metastasis. Expression of this cell surface receptor has been used as a prognostic marker for breast cancer.

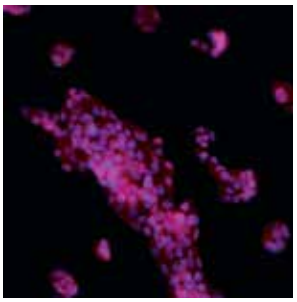
HER4 (ErbB4) protein contains extracellular ligand-binding domains, a transmembrane region, and a cytoplasmic tail containing the tyrosine kinase domain. Upon ligand binding, ErbB4 homo- or hetero-dimerizes with other family members, to become phosphorylated, and can then be cleaved, allowing translocation to the nucleus. The HRF1 antibody recognizes membrane-bound, cytoplasmic, and nuclear ErbB4. Cytosolic ErbB4 expression was found to correlate with a positive prognosis in a subset of breast cancer patients, while nuclear ErbB4 expression was inversely correlated with tumor grade and aggressiveness of breast tumors.

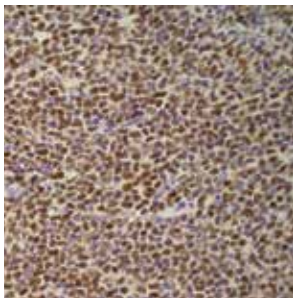
Comparison of isotype control and ErbB4 expression in breast cancer tissue

Immunohistochemistry of formalin-fixed paraffin embedded human ductal carcinoma using 10 ug/mL Mouse IgG2b K Isotype Control eFluor® 570 (left, cat. no. 41-4732) or 10 ug/mL Anti-Human/Mouse ErbB4 (Her4) eFluor® 570 (right, cat. no. 41-9687). Nuclei are stained with DAPI.



Sox11 is a transcription factor belonging to the SOX (sex determining region Y-related HMG [High Mobility Group] Box) family of proteins. Under normal conditions Sox11 is expressed in the developing central nervous system during embryogenesis and later plays a role in neuronal maturation and epithelial-mesenchymal interactions and is required for the neuronal and mesenchymal progenitor cell survival. Sox11 is expressed in mantle cell lymphoma (MCL) and in subsets of hairy cell leukemias, Burkitt lymphomas, and B cell lymphoblastic leukemias, but is not expressed in other B cell lymphomas or in normal B lymphocytes. Sox11 is also expressed in epithelial ovarian cancer and gliomas.





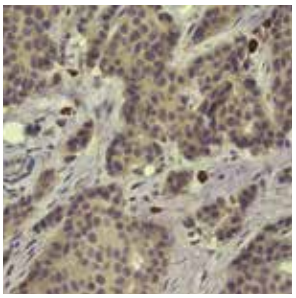
Immunofluorescent detection of Sox11

Immunocytochemistry of fixed and permeabilized HepG2 cells using 10 ug/mL Anti-Human Sox11 Purified (cat. no. 14-9773) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue), colocalization of signal appears pink.

Colorimetric detection of Sox11

Immunohistochemistry of formalin-fixed paraffin embedded human mantle cell lymphoma tissue stained with 10 ug/mL Anti-Human Sox11 Purified (cat. no. 14-9773) followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, with DAB visualization. Nuclei are counterstained with hematoxylin.

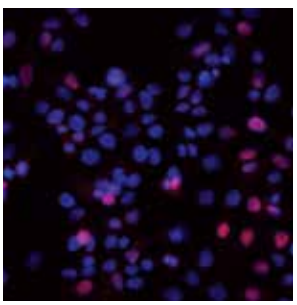
Estrogen Receptor β (ER β) is a member of a superfamily of nuclear receptors, which are ligand-dependent transcription factors. ER β, like ER α, becomes activated by binding the hormone estrogen, causing a dissociation from chaperone proteins and subsequent dimerization with either ER β or ER α. Unlike ER α, the result of ER β signaling is thought to be anti-proliferative and proapoptotic.

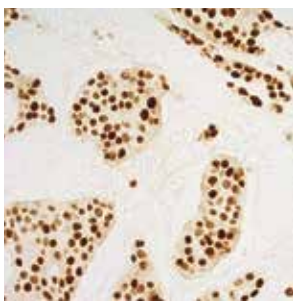


Expression of Estrogen Receptor β in ductal epithelial cells in breast cancer tissue

Estrogen Receptor β is detected in formalin-fixed paraffin embedded human-infiltrating ductal carcinoma, localized primarily to the cytoplasm using 30 ug/mL Anti-Human Estrogen Receptor β Purified (cat. no. 14-9336), followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, with DAB visualization. Nuclei are counterstained with hematoxylin.

Progesterone Receptor (PgR) is a member of a superfamily of nuclear receptors, which are ligand-dependent transcriptional regulators. The human progesterone receptor (PgR) exists in alpha and beta forms, which are expressed at similar levels and predominately form heterodimers. Progestin binding to PgR, allows dissociation of bound chaperone proteins and subsequent dimerization with either PgRa or PgRb. Following activation, dimerized PgR can directly bind to DNA through PREs (progestin response elements) leading to chromatin remodeling and subsequent down regulation or transcription of the target gene. PgR plays a key role in controlling gene expression in breast, uterine, brain, and cardiovascular tissue during development. PgR expressing in breast tissue is indicative of improved survival and a better response to endocrine therapy. In breast and endometrial cancer progression, a predominance of either the alpha or beta form occurs, suggesting dysregulation in the PgRa:PgRb ratio is an early event in cancer. (In cases of ductal carcinoma *in situ* and invasive ductal carcinoma, there is predominance of the alpha form while in uterine cancer a loss of either form is common.)





Immunofluorescent detection of Progesterone Receptor in human breast adenocarcinoma cells

Immunocytochemistry of fixed and permeabilized MCF7 cells using 2.5 ug/mL Anti-Human Progesterone Receptor Purified (cat. no. 14-9764) followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue), colocalization of signal appears pink.

Colorimetric detection of Progesterone Receptor

Immunohistochemistry of formalin-fixed paraffin embedded human infiltrating ductal carcinoma tissue stained with 2.5 ug/mL Anti-Human Progesterone Receptor Purified (cat. no.14-9764) followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, with DAB visualization.

Cancer antibodies listed by format								
Description	Clone	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Human/Mouse Activation-Induced Cytidine Deaminase (AID)	mAID-2	14-5959	13-5959					
Anti-Human AHR	FF3399	14-9854						
Anti-Human AIRE	TM-724	14-9534	13-9534			41-9534		
Anti-Mouse AIRE	5H12	14-5934			53-5934			50-5934
Anti-Human/Mouse Blimp1	6D3	14-5963						
Anti-Mouse BP-1	6C3	14-5891						
Anti-Human Cathepsin L	33-2	BMS1032						
Anti-CCL2 (MCP-1)	2H5	14-7096						
Anti-Human CCL2 (MCP-1)	5D3-F7	14-7099						
Anti-Human Chorionic Gonadotropin	FB12	14-6508		53-6508				
Anti-Human Chorionic Gonadotropin β Subunit	FBT11	14-9872						
Anti-Mouse DLL1 (δ-like 1)	HMD1-5	14-5767						
Anti-Human EGFR-2 (HER-2)	MJD2	14-9757				41-9757		
Anti-Mouse Embigin	G7.43.1	14-5839						
Anti-Mouse Endomucin	eBioV.7C7	14-5851						
Anti-Human/Mouse EOMES	21Mags8	14-4876						
Anti-Mouse Ephrin B1	25H11	14-5300						
Anti-Human/Mouse ErbB4 (Her4)	HFR1	14-9687	13-9687			41-9687		
Anti-Mouse ESAM	1G8	14-5852						
Anti-Mouse F4/80 Antigen	BM8	14-4801						
Anti-Mouse Fc epsilon Receptor Iβ	MAR-1	14-5898						
Anti-Human/Mouse FOXJ1	2A5	14-9965						
Anti-Human Foxp1	JC12	14-9962						
Anti-Human Foxp3	hFOXY	14-5779						
Anti-Human Foxp3	PCH101	14-4776	13-4776	11-4776				
Anti-Human Foxp3	236A/E7	14-4777	13-4777		53-4777			
Anti-Human/Mouse Foxp3	eBio7979	14-7979						
Anti-Mouse Foxp3	NRRF-30	14-4771						
Anti-Mouse/Rat Foxp3	FJK-16s	14-5773	13-5773	11-5773		41-5773	42-5773	
Anti-Human Galectin-3	M3/38	BMS1043						
Anti-Human/Mouse Galectin-3	eBioM3/38	14-5301						
Anti-Rat gamma δTCR	V65	14-5810						
Anti-Human/Mouse Gata-3	TWAJ	14-9966						
Anti-Human/Mouse GILZ	CFMKG15	14-4033						
Anti-Human/Mouse GL7	GL-7	14-5902						
Anti-Glial Fibrillary Acidic Protein	GA5	14-9892			53-9892		42-9892	50-9892
Anti-Mouse GM-CSF	MP1-22E9	14-7331						
Anti-Rat Granulocyte Marker	HIS48	14-0570						
Anti-Human Granulysin PE	eBioDH2	12-8828						
Anti-Human Granzyme B	496B	14-8889					42-8889	
Anti-Human/Mouse HEV Marker	MECA-79				53-6036			
Anti-HIF-1 α	ESEE122	14-9100						
Anti-Human HLA-ABC	W6/32	14-9983						
Anti-Human HLA-DR	LN3	14-9956						
Anti-Human HLA-G	87G	14-9957						
Anti-Human/NHP IFN γ	MD-1	14-7317						
Anti-Human IL-10	JES3-9D7	14-7108						
Anti-Human IL-17A	eBio64CAP17	14-7178						
Anti-Human IL-17A	eBio64DEC17	14-7179						
Anti-Human/Mouse IL-20 Receptor 2	20RNTC	14-1206						
Anti-Human MALT1	50	14-9961						
Anti-Human Mature Macrophage Marker	eBio25F9	14-0115						
Anti-Human MHC Class I free chain	A4	14-9958						
Anti-Rat MHC Class II	HIS19	14-0920						
Anti-Human MICA/B	6D4	14-5788						
Anti-Human/Mouse mTOR	F11	14-2190						
Anti-Mouse/Rat MULT1 (NKG2D Ligand)	5D10	14-5863						
Anti-Human Myeloperoxidase (MPO)	MPO455-8E6	14-1299						
Anti-Human/Mouse Notch1	mN1A	14-5785						
Anti-Mouse OVA257-264	eBio25-D1.16	14-5743						

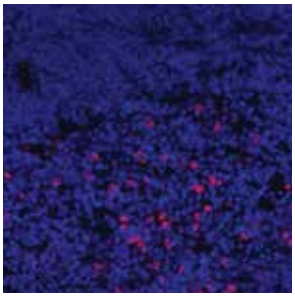
Cancer antibodies listed by format								
Description	Clone	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Human P-Cadherin	12H6	14-9873						
Anti-Pan Cytokeratin (AE1/AE3)	AE1/AE3				53-9003	41-9003	42-9003	50-9003
Anti-Human/Mouse Pax5	1H9	14-9918						
Anti-Human Perforin	J116	14-9989						
Anti-Human Perforin	dG9 (δG9)	14-9994						
Anti-Human Podoplanin	4D5aE5E6	BMS1105						
Anti-Mouse Podoplanin	eBio8.1.1	14-5381	13-5381					
Anti-Human/Mouse PU.1	phpu13	14-9819						
Anti-Human Runx3	R3-5G4	14-9817	13-9817					50-9817
Anti-Mouse Siglec H	eBio440c	14-0333						
Anti-Human SPI-B	235D	14-9790	13-9790					
Anti-Human/Mouse T-bet	eBio4B10	14-5825						
Anti-Mouse TCR beta	H57-597	14-5961						
Anti-Mouse TER-119	TER-119	14-5921						
Anti-Mouse TLR4/MD-2 Complex	MTS510	14-9924	13-9924					
Anti-Mouse TNF alpha	MP6-XT22	14-7321						
Anti-Human TNF-beta	LTX-22	BMS1046						
Anti-Human TNF beta	LTX-21	BMS105						
Anti-Human TRA-1-60 (Podocalyxin)	TRA-1-60	14-8863						
Anti-Human TRA-1-81 (Podocalyxin)	TRA-1-81	14-8883						
Anti-Human TSLP Receptor	eBio1A6	14-5499						
Anti-Human VEGF-R1/FLT-1	Flt-19	BMS196						
Anti-Mouse VEGF Receptor 3	AFL4	14-5988						
Anti-Human CD1a	HI149	14-0019						
Anti-Human CD1b	eBioSN13	14-0018						
Anti-Human CD1c	Flt-19	11-0015		11-0015				
Anti-Human CD1d	51.1	14-0016						
Anti-Human/NHP CD2	RPA-2.10	14-0029						
Anti-Mouse CD2	RM2-5	14-0021						
Anti-Mouse CD3e	145-2C11	14-0031	36-0031	11-0031				
Anti-Mouse CD3e	eBio500A2	14-0033						
Anti-Human CD5	UCHT2	14-0059	13-0059					
Anti-Mouse CD5	53-7.3	14-0051						
Anti-Mouse CD6	IM348	14-0061						
Anti-Mouse CD8b	eBioH35-17.2	14-0083						
Anti-Rat CD8b	eBio341	14-0080						
Anti-Human CD9	eBioSN4	14-0098						
Anti-Mouse CD9	eBioKMC8	14-0091						
Anti-Human CD10	SN5c	13-0108						
Anti-Human CD11b	ICRF44	14-0118						
Anti-Human CD11b (Mac-1α)	LM2/1	BMS104						
Anti-Human CD13	WM-15	14-0138						
Anti-Human CD14	61D3	14-0149						
Anti-Human CD20	L26	14-0202			53-0202		42-0202	50-0202
Anti-Human CD20	2H7	14-0209	13-0209					
Anti-Mouse CD20	AISB12	14-0201						
Anti-Mouse CD21/CD35	eBio8D9	14-0211						
Anti-Human CD23	EBVCS2	14-0238		11-0238				
Anti-Mouse CD23	B3B4	14-0232						
Anti-Mouse CD24	M1/69	14-0242						
Anti-Human/Mouse CD27	LG.3A10	14-0272						
Anti-Human CD34	4H11	14-0349						
Anti-Mouse CD34	RAM34	14-0341						
Anti-Human CD39	eBioA1	14-0399						
Anti-Mouse CD39	24DMS1	14-0391						
Anti-Human CD47	2D3	14-0478						
Anti-Human CD47	B6H12	14-0479		11-0479				
Anti-Mouse CD47	miap301	14-0471						
Anti-Human CD64 (Fc γ Receptor 1)	10.1	14-0649						
Anti-Human CD69	FN50	14-0699						
Anti-Mouse CD69	H1.2F3	14-0691						

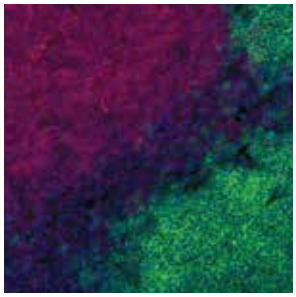
Cancer antibodies listed by format								
Description	Clone	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Mouse CD79a	24C2.5	14-0791						
Anti-Human CD79b	CB3-1	14-0793						
Anti-Mouse CD80 (B7-1)	16-10A1	14-0801						
Anti-Human CD90 (Thy-1)	eBio5E10	14-0909						
Anti-Mouse CD90 (Thy-1)	G7	14-0901						
Anti-Mouse/Rat CD90.1 (Thy-1.1)	HIS51	14-0900						
Anti-Mouse CD90.2 (Thy-1.2)	53-2.1	14-0902						
Anti-Mouse CD90.2 (Thy-1.2)	30-H12	14-0903						
Anti-Mouse CD93 (AA4.1)	AA4.1	14-5892						
Anti-Human CD94	DX22	14-0949						
Anti-Human CD101	BB27	14-1019						
Anti-Human CD104 (Integrin β 4)	B-Ly7	14-1038						
Anti-Mouse CD105 (Endoglin)	439-9B	14-1049	13-1049					
Anti-Human CD117 (c-Kit)	YB5.B8	14-1179						
Anti-Mouse CD117 (c-Kit)	ACK2	14-1172						
Anti-Mouse CD133 (Prominin-1)	13A4	14-1331						
Anti-Human CD134 (OX40)	ACT35	14-1347		11-1347				
Anti-Mouse CD134 (OX40)	OX-86	14-1341						
Anti-Mouse CD153	RM153	14-1531						
Anti-Mouse CD154 (CD40 Ligand)	MR1	14-1541						
Anti-Human CD157	eBioSY11B5	14-1579						
Anti-Human CD200	OX104	14-9200						
Anti-Mouse CD200	OX90	14-5200						
Anti-Mouse CD201 (EPCR)	eBio1560	14-2012	13-2012					
Anti-Mouse CD202b (TIE2)	TEK4	14-5987						
Anti-Human CD205	MG38	14-2059						
Anti-Mouse CD205	205yekta	14-2051						
Anti-Mouse CD207 (Langerin)	eBioRMUL.2	14-2073						
Anti-Mouse CD207 (Langerin)	eBioL31	14-2075	13-2075					
Anti-Mouse CD209a (DC-SIGN)	MMD3				53-2094			50-2094
Anti-Mouse CD209b (SIGN-R1)	eBio22D1	14-2093						
Anti-Human CD227 (Mucin 1)	SM3	14-9893			53-9893		42-9893	
Anti-Human CD244	eBioC1.7	14-5838						
Anti-Mouse CD244.2 (2B4)	eBio244F4	14-2441						
Anti-Human CD268 (BAFF Receptor)	8A7	14-9117						
Anti-Mouse CD268 (BAFF Receptor)	eBio7H22-E16	14-5943						
Anti-Mouse CD273 (B7-DC)	TY25	14-5986						
Anti-Human CD274 (B7-H1)	MIH1	14-5983						
Anti-Mouse CD274 (B7-H1)	MIH5	14-5982						
Anti-Human CD275 (B7-H2)	MIH12	14-5889	13-5889					
Anti-Human CD278 (ICOS)	ISA-3	14-9948						
Anti-Mouse/Rat CD278 (ICOS)	C398.4A	14-9949						
Anti-Human CD279 (PD-1)	eBioJ105	14-2799						
Anti-Human CD279 (PD-1)	MIH4	14-9969						
Anti-Mouse CD279 (PD-1)	J43	14-9985						
Anti-Mouse CD309 (FLK1)	Avas12a1	14-5821						
Anti-Human CD314 (NKG2D)	1D11	14-5878						
Anti-Human CD314 (NKG2D)	5C6	14-5879						
Anti-Human CD325 (N-Cadherin)	8C11	14-3259						
Anti-Human CD357 (AITR/GITR)	eBioAITR	14-5875						

Immune cell markers

Evaluating the host response to cancer

Increased infiltration of immune cells into epithelial tumors can result in physical destruction of carcinomas and improved patient prognosis, although it has also been documented that they facilitate tumor stem cell proliferation and dissemination. Primary infiltrating immune cells include cytotoxic T-lymphocytes (CTL), natural killer cells (NK), leukocytes, macrophages, and mast cells. Regulatory T cells (Tregs) play an important role in suppressing self-antigens, preventing autoimmunity. Cytotoxic T cells (Tc) rely on T-cell receptors (TCR), which are regulated by Tregs, to recognize and destroy known antigens. Myeloid-derived suppressor cells (MDSCs) are potent suppressors of T cells, which accumulate in lymphoid organs and tumors. They can be found at very high levels in gastric, colon, breast, and lung tumors, probably functioning to suppress T cell activity within tumors.





Detection of immune cells in mouse spleen tissue

Mouse spleen section stained with 5 ug/mL Anti-Mouse CD11b M1/70 eFluor® 660 (red, cat. no. 50-0112) and DAPI (blue). M1/70-positive macrophage are scattered throughout the spleen.

Mouse spleen section stained with 10 ug/mL Anti-Mouse CD3 eFluor® 660 (17A2) (red, cat. no. 50-0032),10 ug/mL Anti-Human/Mouse CD45R B220 Ra3-6B2 (green, cat. no. 14-0452), and DAPI (blue). A cluster of CD3-positive T cells and a B cell center are visible in the spleen section.

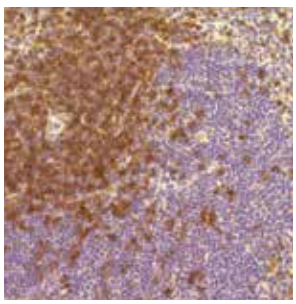
Immune cell markers: eFluor® and Organic Dye formats							
Description	Clone	FITC	Alexa Fluor® 488	eFluor® 520	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Mouse CD3	17A2	11-0032					50-0032
Anti-Mouse CD4	4SM95				41-9766		
Anti-Mouse CD4	RM4-5				41-0042		
Anti-Mouse CD4	RM4-5					42-0042	
Anti-Mouse CD8a	53-6.7					42-0081	50-0081
Anti-Human CD11b	M1/70		53-0112				50-0112
Anti-Mouse CD11c	N418					42-0114	
Anti-Human CD15	HI98					42-0159	
Anti-Human CD45R	PD7/26						50-9458
Anti-Human/Mouse CD45R (B220)	RA3-6B2		53-0452		41-0452	42-0452	50-0452
Anti-Mouse CD68	FA-11						50-0681
Anti-Human CD74	VIC-Y1						50-0747

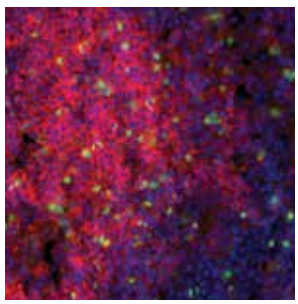
Immune cell markers: Purified and Biotin formats			
Description	Clone	Purified	Biotin
Anti-Mouse F4/80	BM8	14-4801	13-4801
Anti-Human FDC	CNA.42	14-9968	13-9968
Anti-Mouse Ly-6G (Gr-1)	RB6-8C5	14-5931	
Anti-Human Macrophage	HAM56	14-6548	
Anti-Human CD3	UCHT1	14-0038	
Anti-Mouse CD3	17A2	14-0032	
Anti-Rat CD3	eBioG4.18	14-0030	
Anti-Human CD4	CRRY77	14-0045	
Anti-Human CD4	RPA-T4	14-0049	
Anti-Mouse CD4	GK1.5	14-0041	13-0041
Anti-Mouse CD4	4SM95	14-9766	
Anti-Mouse CD4	RM4-5		13-0042
Anti-Mouse CD4	RM4-5	14-0042	
Anti-Rat CD4	OX35	14-0040	
Anti-Human CD8a	AMC908	14-0008	13-0008
Anti-Human CD8a	C8/144B	14-0085	
Anti-Human CD8a	RPA-T8	14-0088	
Anti-Human CD8a	HIT8a	14-0089	
Anti-Mouse CD8a	53-6.7	14-0081	13-0081
Anti-Rat CD8a	OX8	14-0084	
Anti-Human CD11b	ICRF44	14-0118	
Anti-Human CD11b (Mac-1α)	LM2/1	BMS104	
Anti-Mouse CD11b	M1/70	14-0112	13-0112
Anti-Human CD11c	CBR-p150/4G1	BMS112	
Anti-Mouse CD11c	N418	14-0114	
Anti-Human CD11c	3.9	14-0116	
Anti-Human CD15	HI98	14-0159	13-0159
Anti-Mouse CD19	eBio1D3	14-0193	
Anti-Human CD19	HIB19	14-0199	
Anti-Human CD45	CD45-2B11	14-9457	
Anti-Human CD45	HI30	14-0459	
Anti-Mouse CD45	30-F11	14-0451	
Anti-Human/Mouse CD45R (B220)	RA3-6B2	14-0452	
Anti-Rat CD45R (B220)	HIS24	14-0460	
Anti-Human CD45RB	PD7/26	14-9458	
Anti-Mouse CD45RB	C363.16A	14-0455	
Anti-Human/Mouse CD45R (B220)	RA3-6B2	14-0452	
Anti-Rat CD45R (B220)	HIS24	14-0460	
Anti-Human CD56 (NCAM)	5tukon56	14-0565	
Anti-Human CD56 (NCAM)	CMSSB	14-0567	13-0567
Anti-Human CD57	TBO1	14-0577	
Anti-Mouse CD68	FA-11	14-0681	
Anti-Human CD68	eBioY1/82A	14-0689	
Anti-Human CD68	KP1	14-0688	
Anti-Human CD74	VIC-Y1	14-0747	
Anti-Mouse CD86 (B7-2)	GL1	14-0862	
Anti-Rat CD86 (B7-2)	24F	14-0860	

Detection of immune cells in mouse spleen tissue

Formalin-fixed paraffin embedded mouse spleen section stained with 5 ug/mL Anti-Mouse CD4 Purified (cat. no. 14-9766) followed by Anti-Rat IgG Biotin (cat. no. 13-4813), Streptavidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

Frozen section of mouse spleen stained with 10 ug/mL Anti-Mouse/Rat Foxp3 eFluor® 660 (green, cat. no. 50-5773) and 5 ug/mL Anti-Mouse CD4 eFluor® 570 (red, cat. no. 41-0042). Nuclei are stained with DAPI (blue).



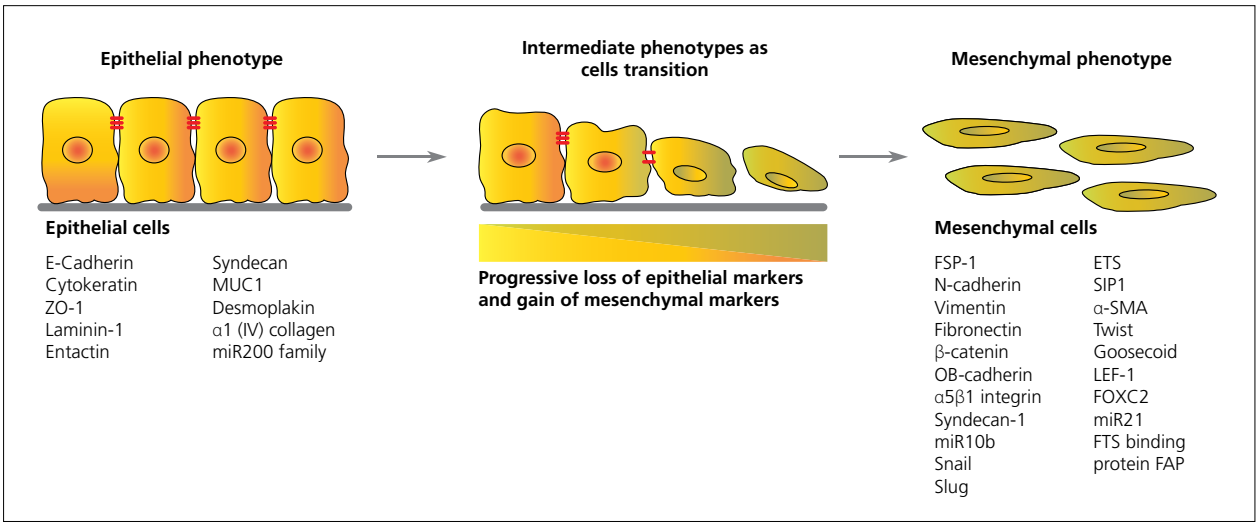


Epithelial to Mesenchymal Transition (EMT)

Characterization of the epithelial to mesenchymal transition

Epithelial to mesenchymal transition (EMT) is a two-step reversible process by which epithelial cells lose their characteristics and acquire those typical of mesenchymal cells. EMT occurs during normal development, cellular repair, and cancer metastasis. Epithelial to mesenchymal transition was initially recognized for the role it played in embryogenesis, mainly through a loss of adhesion, cell polarity, destruction of extracellular matrix (ECM), and upregulation of mesenchymal markers.

Epithelial to Mesenchymal Transition



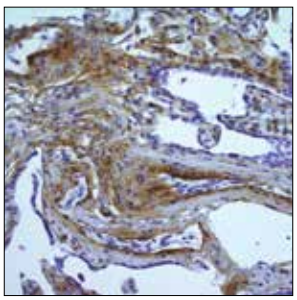
Epithelial phenotype

Epithelial cells line cavity walls in addition to forming the outer covering of the body. The epithelium is supported by the basement membrane, a specialized extracellular matrix (ECM), which separates epithelial cells from connective tissue or stroma. Epithelial-derived tumors are called carcinomas and are much more common than tumors derived from other cell types. One example of a carcinoma is squamous cell carcinoma, characterized by excessive proliferation of cells within the epidermal layer of the skin. In addition to serving as the protective covering of the body, some epithelial cells play a role in the secretion of substances into ducts or cavities. Accumulation of mutations in these types of epithelial cells can give rise to adenocarcinomas. Non-epithelial tissues can also give rise to malignancy and may involve sarcomas derived from mesenchymal cells or hematopoietic malignancies originating in the embryonic mesoderm, in addition to neuroectodermal tumors involving the central and peripheral nervous system.

Mesenchymal phenotype

Mesenchymal cells derive mainly from the mesoderm and a small number from the ectoderm. Mesenchymal cells are typically small spindle-shaped cells that are highly mobile in contrast to epithelial cells. All connective tissue in the body is derived from mesenchymal cells which can differentiate into osteocytes, adipocytes, myocytes, and chondrocytes. Mesenchymal cells can be isolated from multiple tissues including fallopian tubes, fetal liver, lung, cord, and peripheral blood.

Cadherins are proteins which after post translational modification mediate cell to cell adhesion through calcium ions. Cadherin expression is important during development to mediate migration, separation, and positioning of cells and later to maintain tissue structure. The cadherin family is comprised of four groups: classical, desmosomal, protocadherins, and unconventional. The classical group members are named according to their location, epithelial cadherin (E-Cadherin), neural cadherin (N-cadherin), placental cadherin (P-Cadherin), and CD144 vascular endothelial cadherin (VE-Cadherin). Desmosomal cadherins, such as desmoglein, localize to plasma membrane adhesions found in simple and stratified squamous epithelial cells as well as muscle tissue. Disruption of desmosomal structure or function through either genetic or autoimmune mechanisms targeting cadherins can lead to cardiomyopathy or blistering diseases. Protocadherins are mostly found expressed in the developing nervous system and differ from other cadherin family members in that the intracellular domain does not attach to the cytoskeleton.

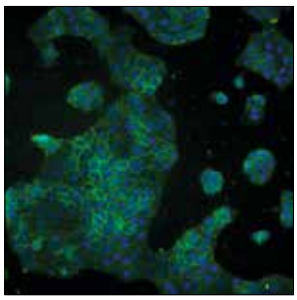


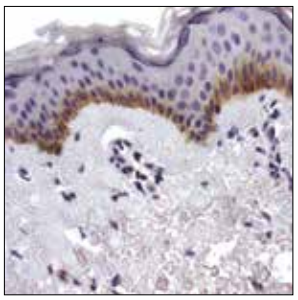
Expression of Collagen type IV in human placenta

Collagen Type IV expression is localized to the extracellular matrix of the placenta. Collagen Type IV is shown in human formalin-fixed paraffin embedded placenta stained with 5 ug/mL Anti-Human Collagen Type IV Purified (cat. no. 14-9871), followed by Anti-Mouse IgG Biotin, Streptavidin HRP, with DAB visualization. Nuclei are counterstained with hematoxylin.

E-Cadherin expression at epithelial cell junctions

E-Cadherin's cytoplasmic domain interacts with α-, β-, and γ-catenins and actinins which bind E-Cadherin to the cytoskeleton. E-Cadherin is commonly lost during epithelial to mesenchymal transition. Expression is localized to the membranes of adjoining canine kidney epithelial cells using 20 ug/mL Anti-CD324 (E-Cadherin) Alexa Fluor® 488 (green, cat. no. 53-3249). Nuclei are stained with DAPI (blue).



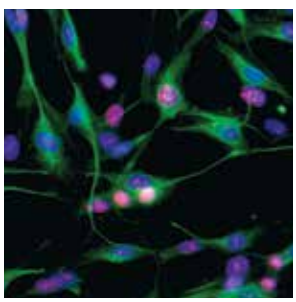


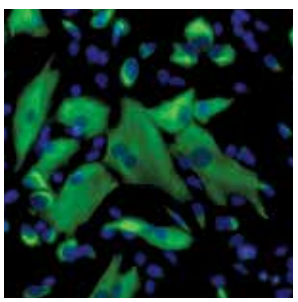
P-Cadherin expression in human skin

P-Cadherin expression is localized to the basal layer of the epidermis. P-Cadherin is visualized using 10 ug/mL Anti-Human P-Cadherin Purified (cat. no. 14-9873), followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

Vimentin expression in proliferating glioma cells

Dividing cells are visualized here by ICC staining of fixed, permeabilized C6 rat glioma cells using 10 ug/mL Anti-Mouse/Rat Ki-67 eFluor® 660 (red, cat. no. 50-5698). Intermediate filaments are stained with 1 ug/mL Anti-Vimentin FITC (green, cat. no. 11-9897). Nuclei are counterstained with DAPI (blue). Nuclei of dividing cells co-express Ki-67 and DAPI and appear pink.





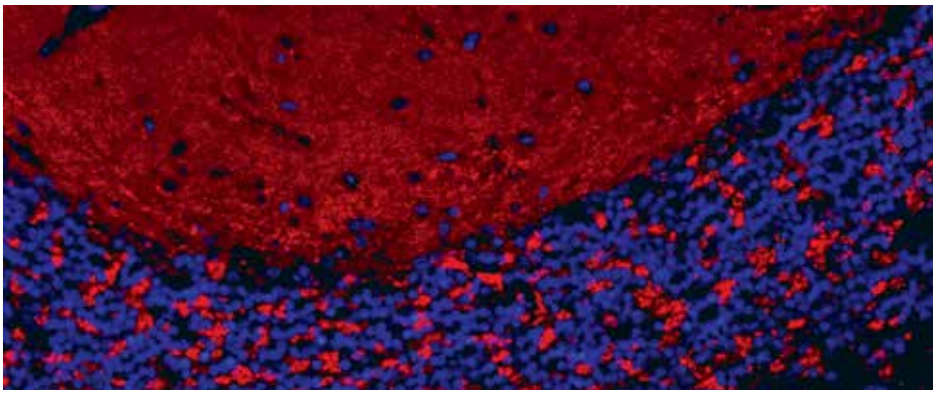
Expression of Vimentin in human neuroblastoma cells

Vimentin, an intermediate filament expressed in subsets of progenitor cells, visualized by immunocytochemical staining of fixed, permeabilized SK-N-SH human neuroblastoma cells using 1 ug/mL Anti-Vimentin FITC (cat. no. 11-9897). Nuclei are counterstained with DAPI.

Stem cells

Stem cells are characterized as self-renewing and multipotent with the potential to remain undifferentiated and thus renew the stem cell niche population, or are capable of differentiating into cell types of more restricted lineage. Stem cell research has encompassed the three main types of stem cells: embryonic stem cells (ESC), adult stem cells, and induced pluripotent stem cells. ESC are totipotent self-renewing cells derived from the inner cell mast of the blastocyst, which can divide indefinitely and differentiate into the three germinal layers; ESCs develop into any cell type found in the body, while adult stem cells are multipotent, self-renewing, tissue-specific stem cells usually only capable of becoming a cell type according to the tissue it is isolated from (for example, a blood stem cell making different types of blood cells). Induced pluripotent stem cells (iPSC) are derived from mature somatic cells that have been reprogrammed using Oct4, Sox2, and KLF4 to assume a pluripotent, self-renewing population of stem cells. iPSC are commonly used for toxicity studies in drug development, disease modeling, and transplant therapy. Antibodies are available for characterization of both undifferentiated and differentiated cell populations in addition to growth factors for stem cell proliferation and induction of differentiation.

The three germinal layers consist of ectoderm, mesoderm, and endoderm. Ectoderm forms the outer germinal layer of the embryo, which differentiates into skin, nervous system, sensory organs, tooth enamel, and eye lens. The mesoderm forms the middle layer of the embryo, from which mesenchymal stem cells (MSC) are derived. MSC are multipotent, self-renewing stem cells capable of differentiating into osteoblasts, chondrocytes or adipocytes. MSCs typically have long, thin cell bodies with a large nucleus and resemble fibroblasts growing as adherent cells *in vitro*. They can be involved in tumor progression by suppressing the host immune response and by promoting angiogenesis in localized tumor areas. Endoderm germinal cells develop in the inner cell layer, differentiating into lungs, digestive organs, liver, and pancreas. Development of endodermal cells is dependent upon transcription factors including SOX and forkhead families.



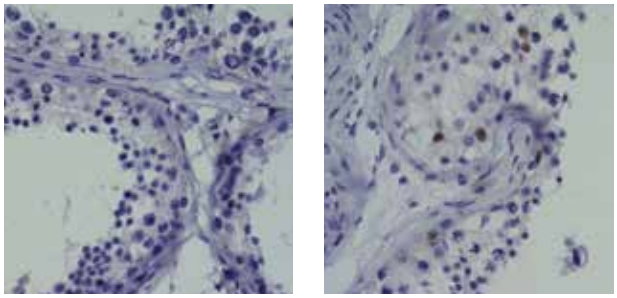
Synaptophysin eFluor® 615

Synaptophysin, also known as Major Synaptic Vesicle Protein p38, is expressed in neuroendocrine tumors and neuroblastomas. It has also been used to characterize mature neurons differentiated from neural stem cells. Synaptophysin is visualized here by immunostaining of formalin-fixed paraffin embedded human cerebellum using Anti-Human Synaptophysin eFluor® 615 (red, cat. no. 42-6525). Nuclei are counterstained with DAPI (blue).

Sox2 transcription factor belongs to the SOX (sex-determining region, Y-related, high mobility group [HMG] box) family of proteins. Sox family members play a role in early organ development, particularly Sox2, which is essential for regulating genes controlling normal mammalian embryogenesis. Sox2 and family member Sox3 are expressed as early as the preimplantation and epiblast stages, while later expression is restricted to the neuroepithelium. Sox1 expression coincides with the induction of neural ectoderm, and by early gastrulation all three genes are expressed throughout the cells comprising the neural plate.

Detection of Sox2 in human testes

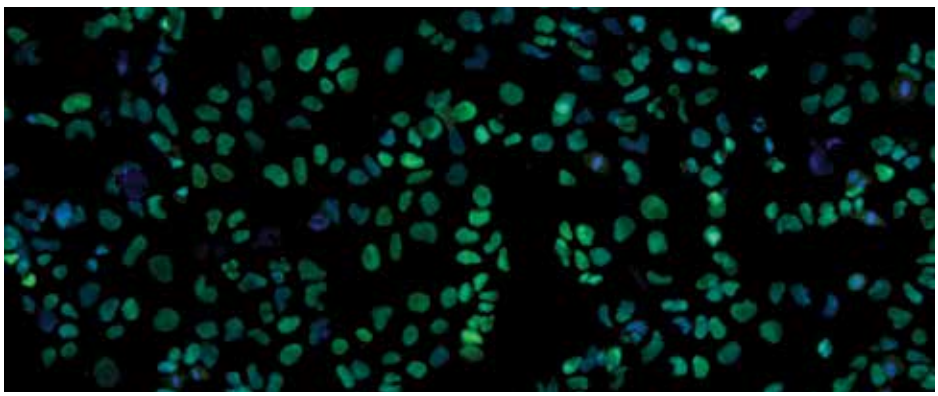
Sox2 expression is localized to the nucleus of Sertoli cells in human testes tissue. Immunohistochemistry of formalin-fixed paraffin embedded human testes tissue using low-pH antigen retrieval (cat. no. 00-4955) with 5 ug/mL Rat IgG2a K Purified (cat. no. 14-4321) (left) or 5 ug/mL Anti-Human/Mouse Sox2 Purified (right) followed by Anti-rat IgG Biotin, Streptavidin HRP and DAB visualization. Nuclei are counterstained with hematoxylin.



Sox2 is necessary for maintaining self-renewal and pluripotency of embryonic stem cells (ESC). Oct4 (POU5F1), KLF4, c-myc, and Sox2 were the original four factors used to reprogram differentiated mouse and human cells into induced pluripotent stem cells (iPSC). Sox2, in combination with Nanog and POU5F1, is expressed in the inner cell mass of the blastocyst and acts cooperatively to maintain pluripotency in both mouse and human embryonic stem cells. Expression of Sox2 is tightly regulated, with recent studies demonstrating small changes in embryonic stem (ES) cell levels of Sox2, which can trigger differentiation into multiple cell types. Expression is not limited to ES cells but is also essential for early neurogenesis where expression becomes restricted to the neural plate, and later to neural stem cells, functioning to suppress neural differentiation. Sox2, in combination with other stem cell markers, can be used to characterize multiple stem cell populations. Ectopic expression of Sox2 has been associated with multiple cancer types, including colorectal and breast.

Nuclear expression of Sox2 in neural progenitors

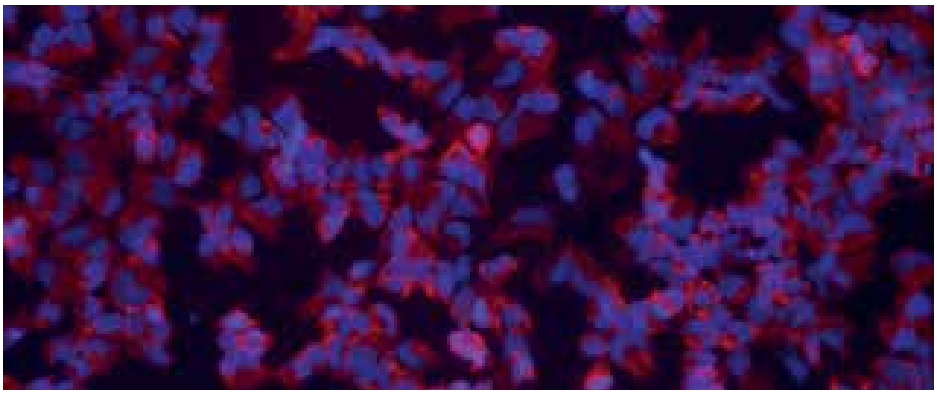
Fixed and permeabilized human NTera2 neural progenitors are stained with 10 ug/mL Anti-Human/Mouse Sox2 Alexa Fluor® 488 (green, cat. no. 53-9811), a nuclear transcription factor involved in early neurogenesis. Nuclei are counterstained with DAPI (blue), colocalization appears aqua.



Sox10 SRY-related HMG-box contains a DNA-binding domain (high-mobility group domain) and functions as a transcription factor in neural and glial precursors, multipotent vascular stem cells, melanomas, and gliomas.

The Musashi family of RNA-binding proteins is comprised of Musashi-1 and Musashi-2, which are expressed at high levels in ectodermal and epithelial stem cells, tumor cells, and specifically neural stem cells.

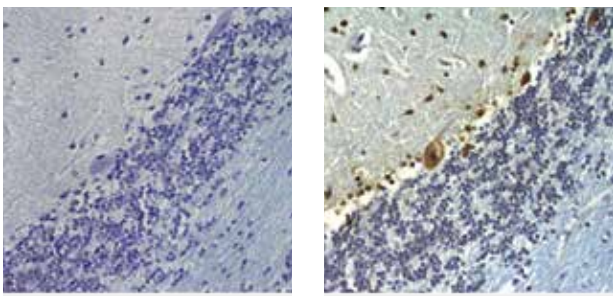
Musashi-1 (Msi1) regulates expression of the NOTCH1 antagonist NUMB by binding RNA sequences containing 5'-GUUAGUUAGUUAGUU-3' and 5'-[GA]U1-3AGU-3'. Musashi-1 functions as a translational repressor, directly regulating its target proteins, Numb and p21 (CIP1). It is a 39 kDa RNA-binding protein expressed in CNS stem and progenitor cells with decreased expression in more differentiated cells. Evidence suggests it is also expressed in stem cells in a variety of other tissues such as the gut, stomach, mammary gland, and hair follicles. In addition to normal tissue expression, Musashi-1 expression has been identified in cells from several tumor types, mainly pulmonary and adenocarcinoma, as well as large- and small-cell carcinomas. Musashi-1 may also play a role in the proliferation and maintenance of stem cells in the central nervous system. Recently, expression in ischemic lesions following stroke provides evidence for a role for Musashi-1 in neurogenesis, as well as regulating apoptosis.



Musashi-1 regulates cell fate and binds RNA

Immunocytochemistry of fixed and permeabilized human neural stem cells using 10 ug/ml Anti-Musashi-1 Purified (cat. no. 14-9896) followed by Anti-Rat TRITC (cat. no. 26-4826). Nuclei are counterstained with Hoechst. Data courtesy of Zeng lab at the Buck Institute.

Musashi-2 (Msi2), a highly conserved RNA-binding protein, contains two RNA-recognition domains and is expressed as one of two isoforms of 36 and 37kDa. During neural development, Musashi-2 is expressed in progenitor cells in the ventricular and subventricular zones, while postnatal expression is limited to multiple subsets of neurons and astrocytes in addition to adult stem cell populations. Functionally, Musashi-2 associates with Sox2 and is required for self-renewal of embryonic stem cells. Much like its role in maintaining stemness in neural progenitors, Musashi-2 is expressed in hematopoietic stem cells and plays a similar role in maintaining self-renewal. Musashi-2 has been found to be overexpressed in human myeloid leukemia with higher expression levels correlating to decreased survival. It may serve as a prognostic marker for acute myeloid leukemia (AML).



Musashi-2 in human cerebellum

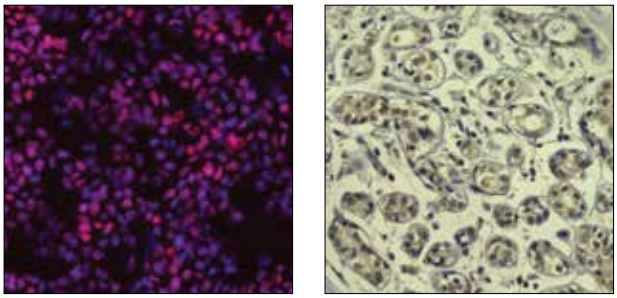
Immunohistochemistry of formalin-fixed paraffin embedded human cerebellum using 10 ug/mL Mouse IgG3 Isotype Control Purified (cat. no. 14-4742) (left) or 10 ug/mL Anti-Human/Mouse Musashi-2 Purified (cat. no. 14-9677) (right) followed by Anti-Mouse IgG Biotin, Streptavidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

Noggin is a secreted, glycosylated, chemokine that binds and inhibits TGFβ (transforming growth factor β) family members such as bone morphogenic protein-4 and -2 (BMP-4, BMP-2). The binding of noggin to BMPs prevents interaction with signal-transducing BMP receptors (BMPR), responsible for activation of VEGF and other pathways that affect vascular patterning, neural tube formation, mesodermal differentiation, and oligodendrogliogenesis. Noggin expression is restricted both temporally and by cell type: for example, by chondrogenic precursor cells during limb development, ectodermal cells in the dorsal neural tube, ependymal cells adjacent to the subventricular zone in adult brain, and stromal cells underlying luminal epithelium.

EZH2 (Enhancer of zeste homolog 2), is a member of the polycomb group (PcG) proteins responsible for gene expression and repression through recognition and modification of histone methylation and chromatin structure. EZH2 comprises the catalytic subunit of the polycomb repressive complex 2 (PRC2) and directly represses gene expression through methylation of histone H3 on lysine 27 (H3K27) as well as direct protein targets, such as GATA4. The PRC2 complex gene repression is necessary for proper development and differentiation. Underexpression of EZH2 is linked to abnormal developmental patterning and a loss of stem cell pluripotency, while overexpression of EZH2 is present in many tumors, correlating with poor prognosis in melanoma, breast, and prostate cancer.

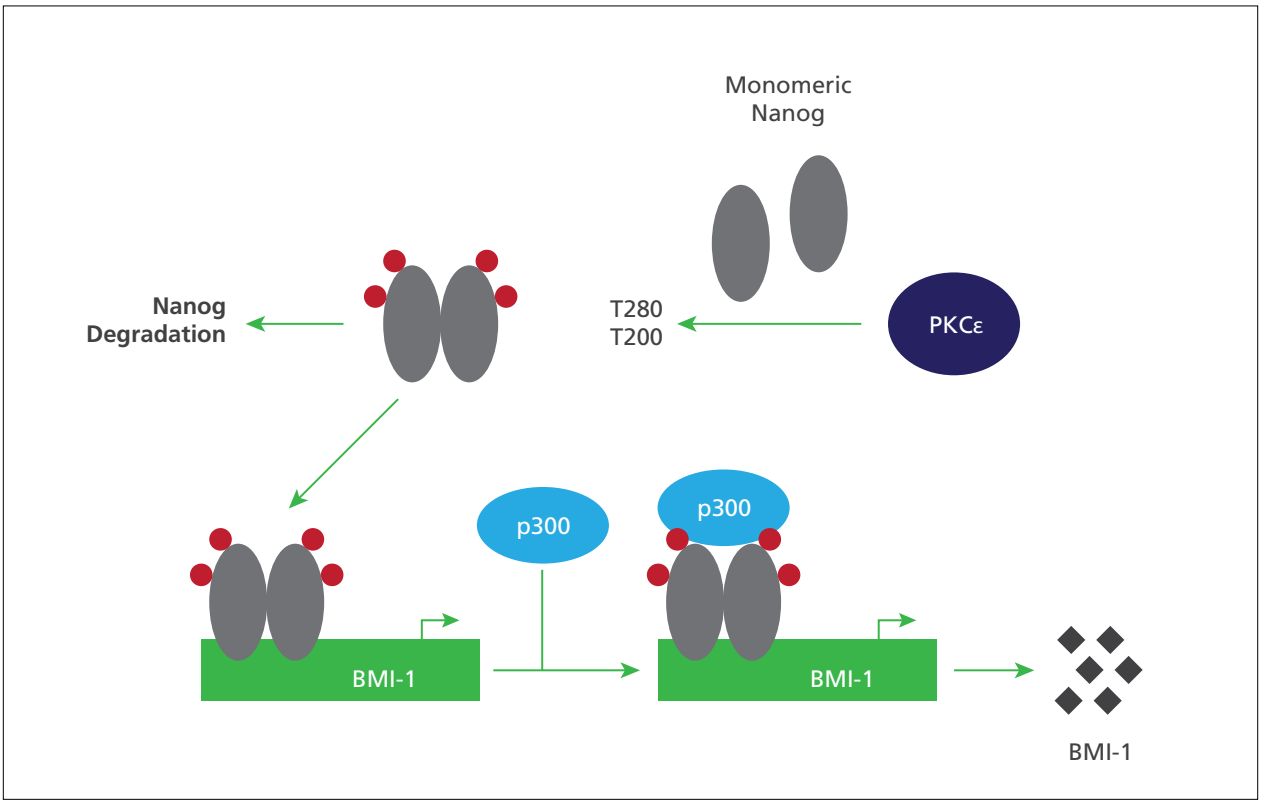
Expression of Nanog in human neural progenitor cells and breast cancer tissue

Immunocytochemistry of fixed and permeabilized human neural progenitor cells using 5 ug/mL Anti-Human Nanog Purified (cat. no. 14-5768), followed by 10 ug/mL F(ab')2 Anti-Mouse IgG eFluor® 570 (red, cat. no. 41-4010). Nuclei are stained with DAPI (blue), colocalization appears pink. Immunohistochemistry of formalin-fixed paraffin embedded human infiltrating ductal carcinoma using 20 ug/mL Anti-Human Nanog Purified (cat. no. 14-5768), followed by Anti-Mouse IgG Biotin (13-4013), Avidin HRP, and DAB visualization. Nuclei are counterstained with hematoxylin.

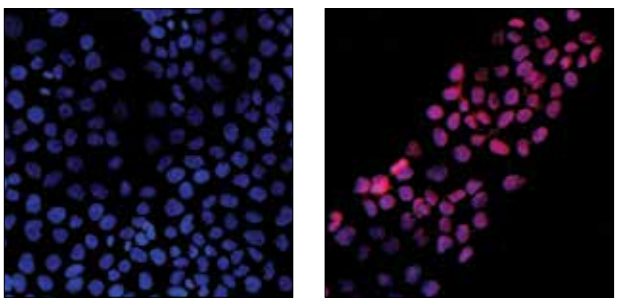


Nanog is a multidomain homeobox transcription factor that has been shown to maintain pluripotency of embryonic stem cells, independent of LIF/Stat3. Expression of Nanog in mouse is specific to early embryos, the inner cell mass of the blastocyst, embryonic stem cells, and embryonic germ (EG) cells. Nanog expression often overlaps, but is not identical to, that of Oct4. Nanog is downregulated upon cellular differentiation and loss of pluripotency, making it a suitable marker in determining the undifferentiated state of stem cells. Nanog acts as a transcriptional activator and has two activation domains in the C-terminus, called CD2 and WR, and one activation domain in the N-terminus, NK2. The CD2 domain is unique to Nanog, whereas the NK2 DNA binding domain is well conserved.

Regulation of Nanog is crucial for BMI-1 expression levels



BMI-1 (B cell–specific Moloney murine leukemia virus integration site 1), is a member of the polycomb group (PcG) of proteins and is responsible for gene expression and repression through recognition and modification of histone methylation and chromatin structure. BMI-1 contains a ring finger domain, a helix-turn-helix domain necessary for inducing telomerase activity, and is a member of the polycomb repressive complex 1 (PRC1). BMI-1 localizes to the nucleus and plays an important role in self-renewal of normal as well as hematopoietic, embryonic, neural, and other stem cell populations. This occurs through the regulation of genes involved in proliferation, such as Sox2 and KLF4. Decreased BMI-1 expression is associated with differentiation, while overexpression is frequently found in a variety of cancers and is associated with poor prognosis. BMI-1 has been shown to be essential for epithelial to mesenchymal transition (EMT) in head, neck, and breast cancers. Elevated BMI-1 expression correlates with poor prognosis in patients with neuroblastoma, glioma, non-Hodgkin B-cell lymphoma, oropharyngeal squamous-cell cancer, and non–small-cell lung cancer, in addition to primary and metastatic melanoma.



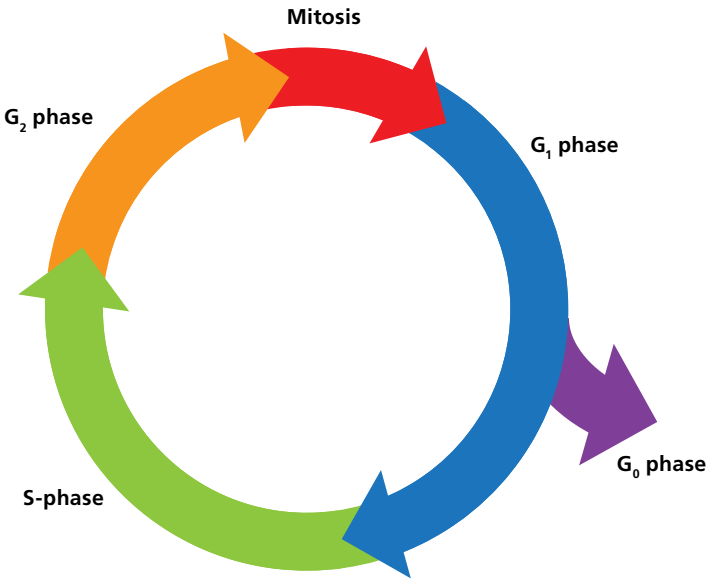
Regulation of gene expression through nuclear localization of BMI-1

Immunocytochemistry of fixed and permeabilized human epithelial carcinoma cells stained with 20 ug/mL of Mouse IgG2a K Isotype Control eFluor® 660 (left, cat. no. 50-4724) or 20 ug/mL of Anti-Human BMI-1 eFluor® 660 (right, cat. no. 50-9702). Nuclei are stained with DAPI (blue), colocalization appears pink.

Stem cell antibodies listed by format								
Description	Clone	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Human α-Fetoprotein	1E8	14-9499	13-9499					
Anti-Human α-Fetoprotein	AFP3	14-6583			53-6583			
Anti-Human Arginase-1	sl6arg	14-9779						50-9779
Anti-Human BMI-1	AF27	14-9702						
Anti-Human Brachyury	X1AO2	14-9770						
Anti-Human Chorionic Gonadotropin	FB12	14-6508			53-6508			
Anti-Human Chorionic Gonadotropin β Subunit	FB11	14-9872						
Anti-Mouse Endomucin	eBioV.7C7	14-5851						
Anti-Mouse Ephrin B1	25H11	14-5300						
Anti-EZH2	AC22	14-9867						
Anti-Human/Mouse Gata-4	eBioEvan	14-9980						
Anti-Human Globo H	VK9	14-9700						
Anti-Human Hes1	4H1HES1	14-9799						
Anti-Human LMO2	1A9-3B11	14-9899						
Anti-Mouse/Rat MASH1	24B72D11	14-5794						
Anti-Musashi-1	14H1	14-9896						
Anti-Human/Mouse Musashi-2	C1	14-9677						50-9677
Anti-Myogenin	F5D	14-5643			53-5643			
Anti-Human Nanog	hNanog.1	14-5769						
Anti-Mouse Nanog	eBioMLC-51	14-5761			53-5761			50-5761
Anti-Human Nestin	10C2	14-9843			53-9843			
Anti-Mouse/Rat Nestin	Rat-401 (Rat401 (4D4))	14-5843						
Anti-Human Neural/Glial Antigen 2 (NG2)	9.2.27	14-6504			53-6504			
Anti-Human Noggin	1H8noggin	14-9009						
Anti-Human/Mouse Notch1	mN1A	14-5785						
Anti-Human/Mouse OCT3/4	EM92	14-5841			53-5841	41-5841		50-5841
Anti-Pax6	AD2.38	14-9914						
Anti-Human Placental Alkaline Phosphatase	8B6	14-9870						50-9870
Anti-Human Pokemon (LRF)	13E9	14-3309						
Anti-Polysialylated Neural Cell Adhesion Molecule	12E3	14-9118						
Anti-Human Snail1	20C8	14-9859			53-9859			
Anti-Human/Mouse Sox2	Btjce	14-9811			53-9811	41-9811		50-9811
Anti-Human/Mouse Sox9	GMPR9	14-9765						
Anti-Sox10	20B7	14-5923						
Anti-Human/Mouse SSEA-1	eBioMC-480	14-8813						
Anti-Human/Mouse SSEA-3	eBioMC-631	14-8833						
Anti-Human SSEA-4	eBioMC-813-70	14-8843				41-8843		50-8843
Anti-Human SSEA-5	8E11-SSEA5	14-8857						
Anti-Stro-1	STRO-1	14-6688						
Anti-Human TAL-1	2TL242	14-9101						
Anti-Human TRA-1-60 (Podocalyxin)	TRA-1-60	14-8863						
Anti-Human TRA-1-81 (Podocalyxin)	TRA-1-81	14-8883						
Anti-Human/Mouse UTF1	MFCDA84	14-9849						
Anti-Human CD9	eBioSN4 (SN4 C3-3A2)	14-0098						
Anti-Mouse CD9	eBioKMC8	14-0091						
Anti-Human CD15	HI98	14-0159	13-0159				42-0159	
Anti-Human CD34	4H11	14-0349						
Anti-Mouse CD34	RAM34	14-0341						
Anti-Human CD38	HIT2	14-0389						
Anti-Human/Mouse CD44	IM7	14-0441						
Anti-Human CD47	2D3	14-0478						
Anti-Mouse CD47	miap301	14-0471						
Anti-Human CD84	B6H12	14-0479		11-0479				
Anti-Human CD84	2G7		13-0849					
Anti-Human CD90 (Thy-1)	eBio5E10	14-0909						
Anti-Mouse CD90 (Thy-1)	G7	14-0901						
Anti-Human CD93	R139	14-0939						
Anti-Mouse CD93 (AA4.1)	AA4.1	14-5892						
Anti-Mouse CD105 (Endoglin)	MJ7/18	14-1051						
Anti-Human CD117 (c-Kit)	YB5.B8	14-1179						
Anti-Mouse CD117 (c-Kit)	ACK2	14-1172						
Anti-Human CD123	6H6	14-1239						
Anti-Mouse CD133 (Prominin-1)	13A4	14-1331						
Anti-Mouse CD144 (VE-Cadherin)	eBioBV14 (BV14)	14-1442						
Anti-Human CD150	A12 (7D4)	14-1509						
Anti-Mouse CD184 (CXCR4)	2B11	14-9991						
Anti-Mouse CD201 (EPCR)	eBio1560	14-2012	13-2012					
Anti-Mouse CD202b (TIE2)	TEK4	14-5987						
Anti-Mouse CD309 (FLK1)	Avas12a1	14-5821						

Cell proliferation

The study of cell proliferation is important in understanding uncontrolled cell growth as a result of cancer, in addition to cell development, regulation, and differentiation. Comprehending the effects of gene addition or deletion and chemical additives on cells can be observed with proliferation assays.



Cell cycle analysis

G_0 phase: Resting cells quiescent

G_1 phase: Cell growth, synthesis of mRNA and proteins

S-phase: DNA replication producing two identical sets of chromosomes

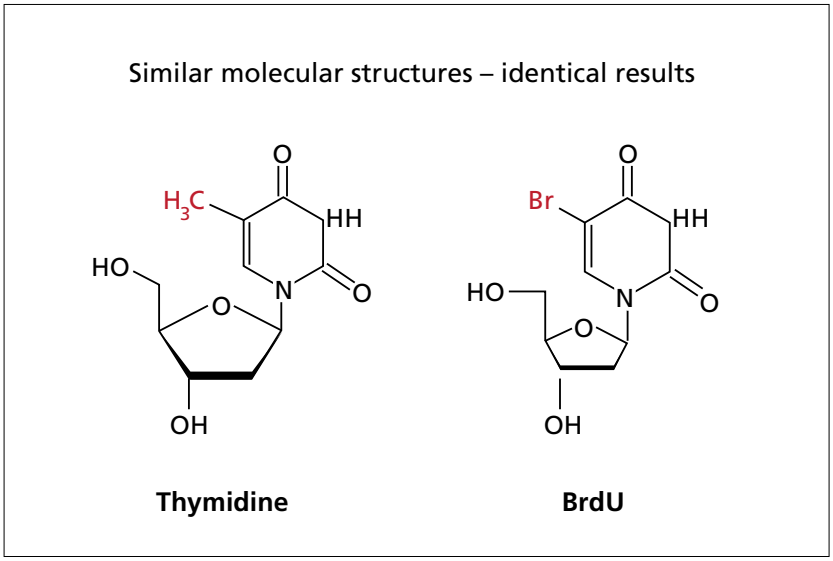
G_2 phase: Cell growth and protein synthesis occurs

Mitosis: The nucleus and cytoplasm divide

Methods to evaluate cell proliferation			
BrdU	Ki-67	PCNA	Proliferation Dyes
Measures cells in S-phase only	Measures proliferating cells at any cell cycle stage except G_0	Measures S-phase but also includes late-G_1 phase	Measures generational proliferation
Pulse-labeling common to avoid cytotoxicity	BrdU is a subset of Ki-67 positive cells	Data supports IHC applications. Not as robust for flow cytometry	Long-term labeling assay. Does not require fixation
In long-term culture, BrdU can be pulse-labeled and washed out. Dividing cells do not incorporate BrdU, so toxicity is limited.	Ki-67 and BrdU are used together in both IHC and flow cytometry		Cannot distinguish cell cycle phases of daughter cells

BrdU

BrdU is a synthetic analog for thymidine, which can be integrated into DNA during S-phase. BrdU incorporation is measured using an Anti-BrdU antibody, showing at least one round of S-phase has been completed. ^{3}H- thymidine-/MTT assays are a sensitive and accurate way to measure overall proliferation, although information is unavailable as to which cells have gone through S-phase.

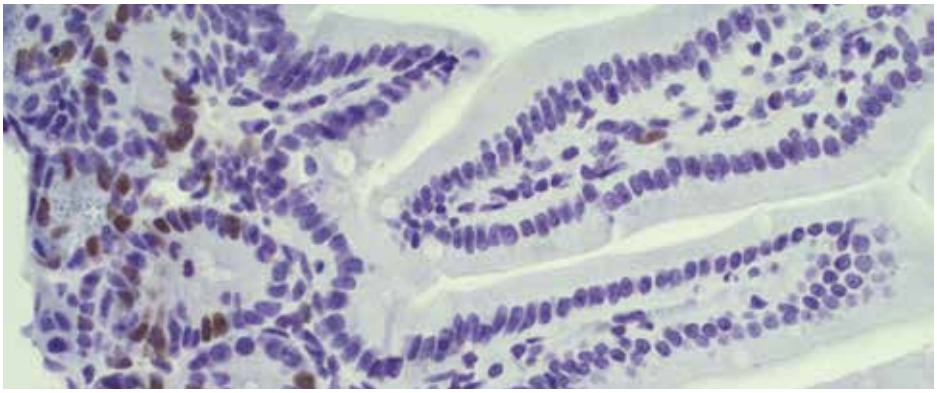


The BrdU Kit for immunohistochemistry (IHC)/immunocytochemistry (ICC) contains the necessary reagents and buffers for identifying and examining proliferating cells by immunohistochemical or immunocytochemical analysis. Cells are labeled *in vitro* or *in vivo* with 5-bromo-2'-deoxyuridine (BrdU), a synthetic analog of thymidine, which is incorporated into DNA in place of thymidine during the S-phase of the cell cycle. Following fixation and antigen retrieval steps, cells or tissue sections are stained for BrdU incorporation and visualized using an Avidin-HRP (horseradish peroxidase) and DAB (3, 3'-Diaminobenzidine) substrate reaction. This kit has been optimized for IHC with both frozen and paraffin embedded BrdU-labeled mouse intestine and ICC of BrdU-pulsed HeLa cells grown on culture slides. The BrdU kit is available in multiple formats.

BrdU kits			
Description	Species	Application	Cat. No
BrdU Kit for IHC/ICC Colorimetric	Chimpanzee, Human, Macaque, Mouse, Rat	IHC- FFPE, IHC- F, ICC	8800-6599
BrdU Kit for IHC/ICC Immunofluorescence eFluor® 570			8841-6599
BrdU Kit for IHC/ICC Immunofluorescence eFluor® 615			8842-6599
BrdU Kit for IHC/ICC Immunofluorescence eFluor® 660			8850-6599

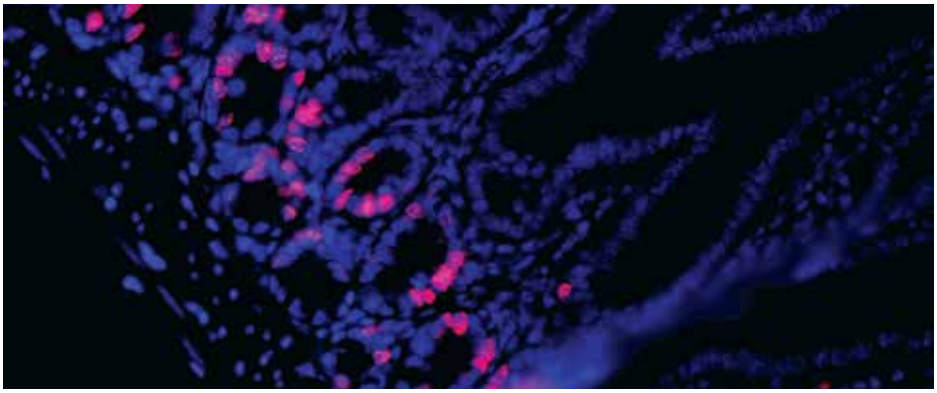
Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

BrdU visualized in cells and tissue



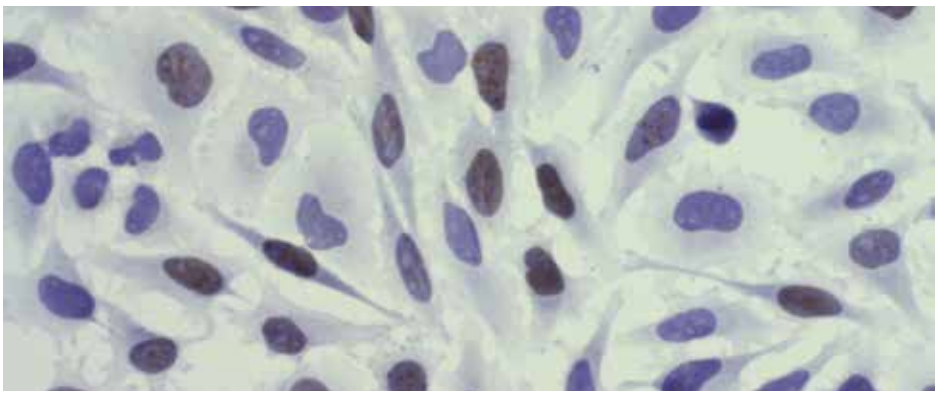
Colorimetric detection of BrdU-positive cells

FFPE BrdU-treated mouse small intestine was stained using the the BrdU Kit for IHC/ICC Colorimetric (cat. no. 8800-6599). Tissues are stained with Anti-BrdU Biotin, followed by Avidin-HRP and DAB visualization. Nuclei are counterstained with hematoxylin.



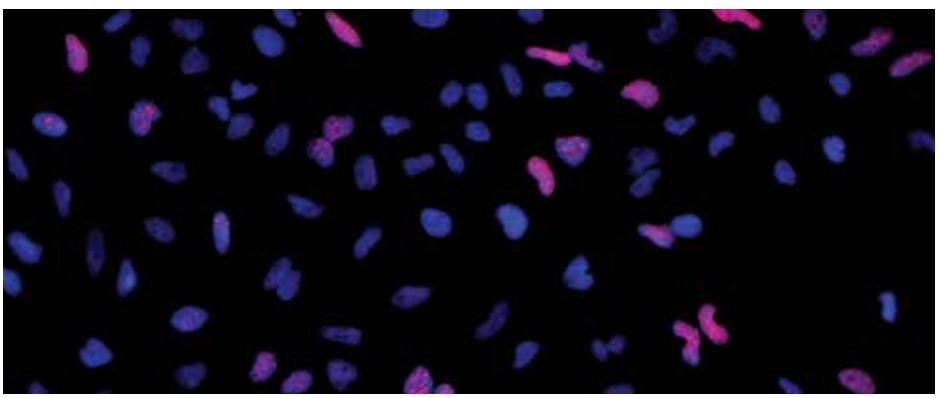
Immunofluorescent detection of BrdU-positive cells

BrdU-treated mouse small intestine was stained using the BrdU Kit for IHC/ICC Immunofluorescence eFluor® 570 (cat. no. 8841-6599). Tissues were stained with Anti-BrdU Biotin, followed by Streptavidin-eFluor® 570. Nuclei are stained with DAPI.



Colorimetric detection of BrdU-positive cells

BrdU-pulsed HeLa cells were stained using the the BrdU Kit for IHC/ICC Colorimetric (cat. no. 8800-6599). Cells were stained with Anti-BrdU Biotin, followed by Avidin-HRP and DAB visualization. Nuclei are counterstained with hematoxylin.

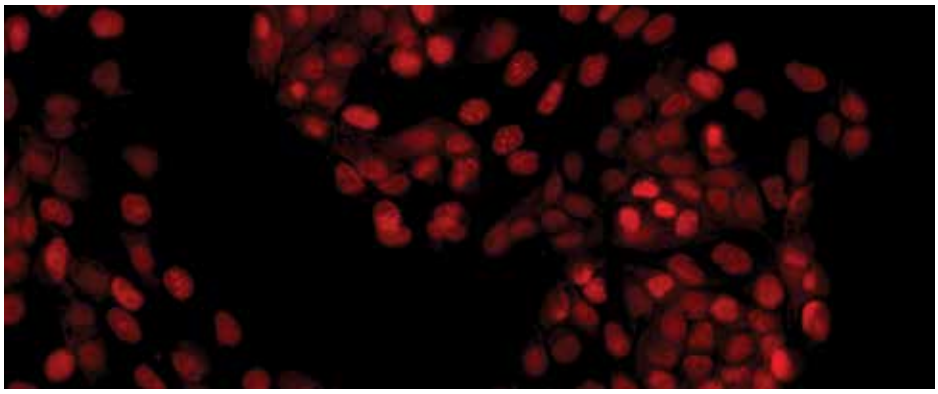


Immunofluorescent detection of BrdU-positive cells

BrdU-pulsed HeLa cells were stained using the the BrdU Kit for IHC/ICC Immunofluorescence eFluor® 615 (cat. no. 8842-6599). Cells were stained with Anti-BrdU Biotin, followed by Streptavidin-eFluor® 615. Nuclei are stained with DAPI.

Peak PCNA expression is detected during S-phase

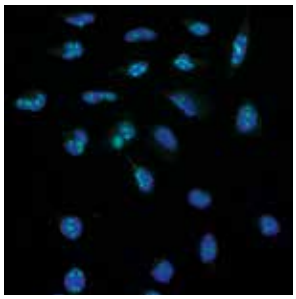
PCNA expression is detected in the nucleus of methanol-fixed canine kidney cells stained with 0.5 ug/mL Anti-Human PCNA eFluor® 615 (red, cat. no. 42-9910).

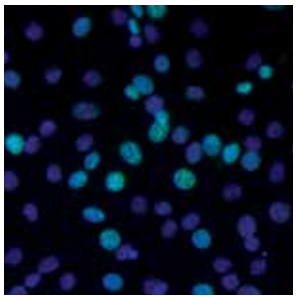


Ki-67 is a nuclear protein present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but absent from resting cells (G_0). Ki-67 is detected within the nucleus during interphase but redistributes to the chromosomes during mitosis. Ki-67 is used as a marker for determining the growth fraction of a given population of cells. In studies of tumor cells, the "Ki-67 labeling index" refers to the number of Ki-67-positive cells within the population, which is then used to predict outcome of particular cancer types. Ki-67 has been shown to interact with the DNA-bound protein chromobox protein homolog 3 (CBX3) (heterochromatin).

Ki-67 expression in HeLa cells

Cervical adenocarcinoma cells were fixed and permeabilized; proliferating cells were stained with 10 ug/mL Anti-Human Ki-67 Biotin (cat. no. 13-5699), followed by 2.5 ug/mL Streptavidin FITC (green). Nuclei are stained with DAPI (blue).





Ki-67 expression in mouse C2C12 myoblast cells

Cells were fixed with formaldehyde and permeabilized; proliferating cells were stained with 5 ug/mL Anti-Mouse/Rat Ki-67 Biotin (cat. no. 13-5698), followed by 2.5 ug/mL Streptavidin FITC (green). Nuclei are stained with DAPI (blue).

Cell cycle antibodies listed by format

Description	Clone	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Anti-Human alpha-Fetoprotein	AFP3	14-6583			53-6583			
Anti-BrdU	BU20A	14-5071						
Anti-Human Cyclin E	HE12	14-9714	13-9714					50-9714
Anti-Human EGFR-2 (HER-2)	MJD2	14-9757				41-9757		
Anti-Human Heat Shock Protein 27	STRSN	14-9112						50-9112
Anti-Human Ki-67	20Raj1	14-5699	13-5699	11-5699		41-5699	42-5699	50-5699
Anti-Mouse/Rat Ki-67	SolA15	14-5698	13-5698	11-5698		41-5698	42-5698	50-5698
Anti-Human LMO2	1A9-3B11	14-9899						
Anti-Human/Mouse Notch1	mN1A	14-5785						
Anti-p21 (WAF1, Cip1) Purified	Polyclonal	14-6715						
Anti-Human PCNA	PC10 (a.k.a. 3F81)	14-9910	13-9910				42-9910	
Anti-Human Plectin	10F6	BMS165						
Anti-Human Skp-2	SJBCH	14-5697						
Anti-Human Trop2 (EGP-1)	MR54	14-6024			53-6024			
Anti-Human CD26	4H3	BMS1023						
Anti-Human CD26	M-A261	BMS143						
Anti-Mouse CD274 (B7-H1)	MIH5	14-5982						
Anti-Human CD274 (B7-H1)	MIH1	14-5983						
Anti-Human CD275 (B7-H2)	MIH12	14-5889	13-5889					
Anti-Human CD325 (N-Cadherin)	8C11	14-3259						

Cell death

Apoptosis

In early-stage apoptosis, the plasma membrane excludes viability dyes such as propidium iodide (PI), 7-AAD, or fixable viability dyes (FVD). These cells will stain with Annexin V due to phosphotidlyserine (PS) present in the inner plasma membrane moving to the outer membrane, distinguishing cells in early apoptosis. However, in late-stage apoptosis, the cell membrane loses integrity, thereby allowing the DNA dyes to enter the interior of the cell. A viability dye can be used to resolve these late-stage apoptotic and necrotic cells (Annexin V, viability dye-positive) from the early-stage apoptotic cells (Annexin V positive, viability dye-negative).

JC-1 is a membrane-permeable dye widely used for determining loss of mitochondrial membrane potential associated with apoptosis or cell stress in flow cytometry and fluorescent microscopy. The dye selectively enters the mitochondria where it reversibly changes color as membrane potentials increase (values more than 80-100 mV). This property is due to the reversible formation of JC-1 aggregates upon membrane polarization, which causes shifts in emitted light from 530 nm (i.e., emission of JC-1 monomeric form) to 590 nm (i.e., emission of J-aggregate) when excited at 488 nm. Both colors can be detected using filters for FITC and PE, respectively. JC-1 is both qualitative, with respect to shift from green to orange fluorescence emission, and quantitative, as measured by fluorescence intensity.

Apoptosis antibodies listed by format					
Description	Clone	Purified	Biotin	FITC	eFluor® 660
Anti-ssDNA (APOSTAIN)	F7-26	BMS156			
Anti-Apoptosis Inducing Factor (AIF) Purified	Polyclonal	14-6050			
Anti-Human Bax	2D2	BMS162			
Anti-Universal Bax	6A7	BMS163			
Anti-Human Bcl-2	Bcl-2/100	14-1028			
Anti-Human Bcl-2	Bcl-2/100	BMS1028		BMS1028FI	
Anti-Human Bcl-2	4D7	BMS1029			
Anti-Mouse Caspase 12	14F7	14-9950			
Anti-Mouse Granzyme B Purified	16G6	14-8822	13-8822		
Anti-Grim-19	1A8	14-9937		11-9937	
Anti-Human Heat Shock Protein 27	STRSN	14-9112			50-9112
Anti-Human Mcl-1	Ab22	14-6701			
Anti-Human NOD2	2D9	14-5869			
Anti-PARP	C2-10	14-6666			
Anti-Human Perforin	dG9 (δG9)	14-9994			
Anti-Mouse PIM-2 Purified	1D12	14-3308			
Anti-Mouse/Rat Receptor Interacting Protein 3 (RIP3)	Polyclonal	14-6048			
Anti-Mouse TNF α	MP6-XT22	14-7321			
Anti-Human TNF-β	LTX-22	BMS1046			
Anti-Human TNF-β	LTX-21	BMS105		BMS105FI	
Anti-Ubiquitin	eBioP4D1 (P4D1)	14-6078			
Anti-XIAP Purified	Polyclonal	14-6047			
Anti-Human/Mouse CD27	LG.3A10	14-0272			
Anti-Human CD30	Ber-H2	14-0309			
Anti-Human CD95 (APO-1/Fas)	APO-1-1	BMS151	BMS151BT	BMS151FI	
Anti-Human CD95 (APO-1/Fas)	DX2	14-0959			
Anti-Human CD180 (RP105)	MHR73-11	14-1809			
Anti-Human CD261 (DR4)	DJR1	14-6644			
Anti-Human CD262 (DR5)	DJR2-2 (a.k.a. 2-6)	14-909			
Anti-Human CD266 (TWEAK Receptor)	ITEM-1	14-9019			
Anti-Human/Mouse CD266 (TWEAK Receptor)	ITEM-4	14-9018			
Anti-Human CD279 (PD-1)	eBioJ105	14-2799			
Anti-Human CD279 (PD-1)	MIH4	14-9969			
Anti-Mouse CD279 (PD-1)	J43	14-9985			
Anti-Human CD279 (PD-1)	J116	14-9989			
CaspGLOW™ Fluorescein Active Caspase Staining Kit		88-7003-42			
CaspGLOW™ Fluorescein Active Caspase-3 Staining Kit		88-7004-42			
CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit		88-7005-42			
CaspGLOW™ Fluorescein Active Caspase-9 Staining Kit		88-7006-42			
JC-1 Mitochondrial Membrane Potential Dye		65-0851-38			

Cell function

Labeling live cells

Calcium Sensing Dye eFluor® 514

Membrane-permeable dyes, such as Calcium Sensing Dye eFluor® 514 can be used to monitor changes in intracellular free calcium concentrations using fluorescence microscopy, flow cytometry, fluorescence spectroscopy, and fluorescence microplate readers. Calcium Sensor Dye eFluor® 514 enters the cell with an acetoxymethyl (AM) ester group that is cleaved by cellular esterases, yielding a membrane-impermeable dye with a peak emission at ~520 nm when excited with the 488 nm laser. Calcium Sensor Dye eFluor® 514, like Fluo-3 and Fluo-4, is a commonly used dye among the visible light-excitable calcium indicators, but with increased cellular uptake and brightness, even at room temperature.

Indo-1 AM Calcium Sensor Dye

This membrane-permeable dye is used for determining changes in calcium concentrations within the cell using fluorescence microscopy, flow cytometry, fluorescence spectroscopy, and fluorescence microplate readers. Once Indo-1 enters the cell, esterases cleave the AM ester group, yielding a membrane-impermeable dye with a peak excitation wavelength of 346 nm. Unbound Indo-1 has a peak emission at 485 nm. Upon binding calcium, the peak emission shifts down to 410 nm.

Cell monitoring dyes		
Description	Application	Cat. No.
Calcium Sensor Dye eFluor® 514	FC, MIC	65-0859
Indo-1 AM Calcium Sensor Dye	FC, MIC	65-0856
Indo-1 AM Calcium Sensor Dye (UltraPure Grade)	FC, MIC	65-0857

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Calcein AM, Calcein Blue AM, Calcein Violet 450 AM

Calcein labeling dyes cross the cell membrane easily, selectively labeling live cells for analysis by flow cytometry or fluorescent microscopy; however apoptotic and dead cells with compromised cell membranes do not retain Calcein.

Calcein dyes are non-fluorescent until they cross the cell membrane of viable cells and are enzymatically processed by intracellular esterases to their fluorescent, membrane–non-permeable form. Dead cells do not have intact cell membranes and cannot retain the cleaved Calcein dyes, nor do they have active esterases to cleave the Calceins to their fluorescent forms. Co-staining with Annexin V or 7-AAD is recommended to allow the greatest resolution between live and dead/apoptotic cells.

Live cell labeling at a glance							
Description	Format	Utility	Viability	Application	Excitation	Emission	Cat. No.
UV Laser							
Calcein Blue AM	Lyophilized	Membrane–permeable live-cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells	Live cells	FC/ MIC	360 nm	445 nm	65-0855
Violet Laser							
Calcein Violet 450 AM	Lyophilized	Membrane–permeable live-cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells	Live cells	FC/ MIC	408 nm	450 nm	65-0854
Blue Laser							
Calcein AM (UltraPure Grade)	Lyophilized	Membrane–permeable live-cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells For improved resolution of live and dead/apoptotic cells using single-color analysis, Calcein Blue AM or Calcein Violet 450 AM are recommended	Live cells	FC/ MIC	495 nm	515 nm	65-0853

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Cell tracking

CellVue dyes offer a stable method to rapidly label the cell membrane of live cells with lipophilic dyes suitable for microscopy and flow cytometry.

Cell tracking dyes				
Description	Excitation	Emission	Application	Cat. No.
Violet Laser				
CellVue® Lavender Cell Labeling Kit	420 nm	461 nm	FC, ICC, IHC, FA	88-0873
Blue Laser				
CellVue® Jade Cell Labeling Kit	478 nm	508 nm	FC, ICC, IHC, FA	88-0876
Red Laser				
CellVue® Maroon Cell Labeling Kit	647 nm	667 nm	FC, ICC, IHC, FA	88-0870
CellVue® Plum Cell Labeling Kit	652 nm	671 nm	FC, ICC, IHC, FA	88-0871
CellVue® Burgundy Cell Labeling Kit	683 nm	707 nm	FC, ICC, IHC, FA	88-0872
CellVue® NIR780 Cell Labeling Kit	633 nm	776 nm	FC, ICC, IHC, FA	88-0875
CellVue® NIR815 Cell Labeling Kit	786 nm	814 nm	ICC, IHC, FA	88-0874
CellVue® Diluent C			FC, IHC	00-4501

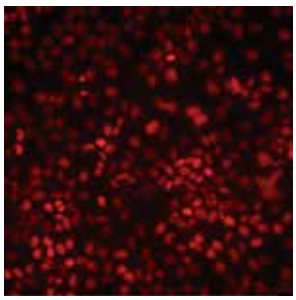
CyTRAK Orange™

CyTRAK Orange™ is an anthraquinone dye with high affinity for double-stranded DNA. It is a membrane-permeable dye that can label live or fixed/dead cells. In flow cytometry, it can be used to distinguish nucleated and non-nucleated cells. In fluorescent microscopy, it can be used to identify and discriminate the nucleus and cytoplasm without the need for a second dye, due to its high-intensity staining of the nucleus and low-intensity staining of the cytoplasm. CyTRAK Orange is optimally excited from 488 to 550 nm, with a peak emission of 610 nm.

DRAQ5™

DRAQ5™ is an anthraquinone dye with high affinity for double-stranded DNA. It is a membrane-permeable dye that can label live or fixed/dead cells. In flow cytometry, this dye can be used to distinguish nucleated and non-nucleated cells. DRAQ5 can also be used to report nuclear DNA content for ploidy and cell cycle analysis because it binds DNA stoichiometrically. In fluorescent microscopy, it can be used as a nuclear counterstain. DRAQ5 can be excited from 488-647 nm, with a peak emission of 670 nm.

CyTRAK Orange™ U2-OS human osteosarcoma cells, counterstained with CyTRAK Orange™ (courtesy of Biostatus).



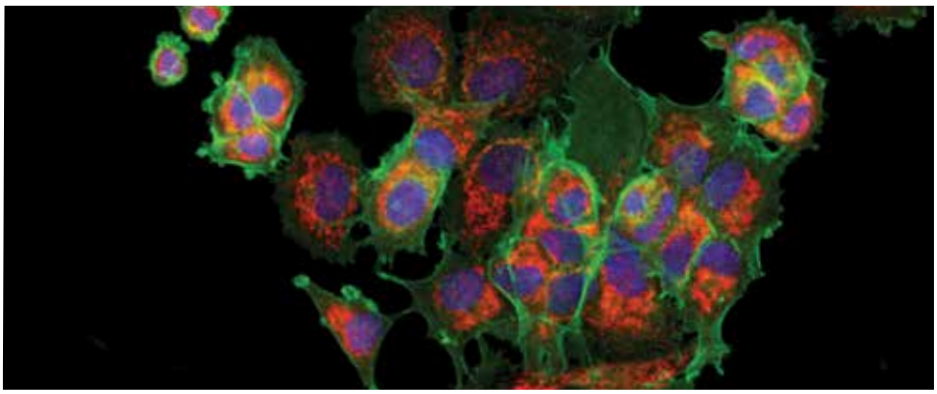
DRAQ5™ Fixed and permeabilized MDCK cells stained with 10 nM DRAQ5™ nuclear stain (cat. no. 65-0880).

Membrane–permeable dyes		
Description	Application	Cat. No
CyTRAK Orange™	FC, ICC, IHC, FA	65-0881
DRAQ5™	FC, ICC, IHC, FA	65-0880

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Secondary reagents

Many options exist for 2- and 3-step staining, from fluorophore-conjugated antibodies to biotinylated secondaries, in addition to Avidin and Streptavidin (SAV) reagents. For 2-step staining it is common to use a purified primary antibody followed by a directly conjugated secondary antibody, which recognizes the host species of the primary antibody. This secondary antibody may be polyclonal and react with all IgG or IgM, or can be specific to the isotype of the primary antibody. For the utmost sensitivity (signal amplification), a 3-step protocol may be optimal, e.g. using a target-specific primary antibody, followed by a biotinylated secondary and subsequently a conjugate.



Multiplex with direct and indirect staining

Mcl-1 recognizes myeloid cell leukemia-1 protein involved in cell proliferation and survival through regulation of Bcl-2 proteins. MCF7 breast adenocarcinoma cells are visualized by ICC staining with 5 ug/mL Anti-Human Mcl-1 Purified followed by Anti-Mouse IgG1 Biotin and Streptavidin eFluor® 570 (red, cat. no. 41-4317). Actin filaments are stained with Phalloidin eFluor® 520 (green, cat. no. 59-6559) and nuclei are counterstained with DAPI (blue).

Selecting the correct secondary antibody

Although directly conjugated antibodies provide a simple and robust assay detection system, there are situations where a primary antibody followed by a secondary antibody is warranted:

- Primary antibody is not available in a conjugated format
- Primary antibody is not available in the desired format
- Amplification of primary antibody signal is needed

Understanding how to choose the appropriate secondary antibody and format is essential for obtaining the best possible staining results.

Application determines the format

Fluorochrome-conjugated secondary antibodies are available in numerous formats, with each specific conjugate determining the application in which the secondary antibody can be used. The variety of available formats provides flexibility, making secondary antibodies versatile across various assay platforms including immunohistochemistry, immunocytochemistry, flow cytometry, immunoassays, and immunoblotting.

Host species and isotype of primary determines the secondary antibody

When selecting a secondary antibody it is important to choose one that recognizes the host species of the primary antibody. For example, a Goat Anti-Mouse IgG can be used with a mouse primary antibody. Secondary antibodies can be either polyclonal, in which case the host species is typically goat or donkey, or monoclonal where the host species is typically mouse or rat. Additionally, secondary antibodies are available that bind to several classes of immunoglobulin, either IgA, IgM, or IgG, in addition to more specific subclasses, such as IgG1, IgG2a, and IgG2b.

Polyclonal antibodies can provide amplification of the signal but must be highly cross-absorbed to provide specificity to the primary antibody being detected and to eliminate reactivity to other species or subclasses of primary antibodies. Most polyclonal antibodies are F(ab')2 fragmented to minimize Fc binding.

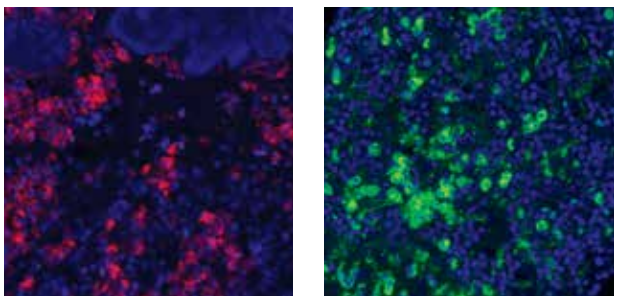
Monoclonal secondaries are typically used when looking at reactivity to a specific IgG subclass. Monoclonal antibodies are consistently reactive to IgG subclasses (IgG1, IgG2a, IgG2b, IgG2c, IgG3), in addition to providing amplification, but to a lesser extent than polyclonal secondaries. Every eBioscience monoclonal antibody has been validated against the specific subclass, as well as lack of reactivity to other subclasses and species. These are useful in multiplexing when using primary antibodies from the same species, but with different subclasses.

Biotin and streptavidin conjugates

Biotin is involved in the metabolism of fatty acids, amino acids, and gluconeogenesis; however, in the laboratory, it can be used to tag molecules of interest for biochemical or cellular studies. While Streptavidin binds to biotin with high affinity, the fluorochrome conjugates are commonly used with indirect staining protocols to detect biotinylated primary antibodies in immunocytochemistry and immunohistochemistry. The Anti-Biotin monoclonal antibody (clone BK-1/39) specifically recognizes biotin and can be used as an alternative to Streptavidin.

IHC using eFluor® 615 and FITC-conjuguated secondary antibodies

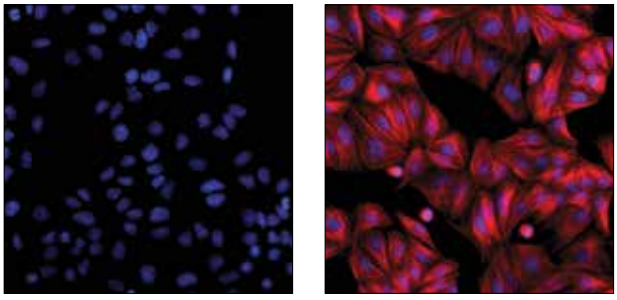
FFPE human tonsil tissue stained with 10 ug/mL Anti-Human CD15 Purified (cat. no. 14-0159), followed by 20 ug/mL Anti-Mouse IgM eFluor® 615 (left, cat. no. 42-5790) or 20 ug/mL Anti-Mouse IgM FITC (right, cat. no. 11-5790) to visualize granulocytes. Nuclei are counter-stained with DAPI.



Secondary reagents listed by format							
Description	Clone	Host	Purified	Biotin	HRP	FITC	TRITC
Anti-Mouse IgG*	Polyclonal	Rat		13-4013			
F(ab')2 Anti-Mouse IgG	Polyclonal	Goat				11-4010	
F(ab')2 Anti-Rabbit IgG	Polyclonal	Goat				11-4938	
Anti-Armenian Hamster IgG	Polyclonal	Goat		13-4113			
Anti-Golden Syrian Hamster IgG	Polyclonal	Goat		13-4213			
Anti-Rat IgG*	Polyclonal	Goat		13-4813			
Anti-Rat IgG	Polyclonal	Goat			18-4818		
Anti-Rat IgG*	Polyclonal	Goat					26-4826
Anti-Human IgM	SA-DA4	Mouse	14-9998	13-9998		13-9998	
Anti-Rat IgG1	R1-3G1	Mouse				11-4814	
Anti-Rat IgG2a	R2a-21B2	Rat		13-4817		11-4817	
Anti-Mouse IgG1*	M1-14D12	Rat		13-4015	18-4015	11-4015	
Anti-Mouse IgG2a	m2a-15F8	Rat		13-4210		11-4210	
Anti-Mouse IgG2b	m2b-25G4	Rat				11-4220	
Anti-Mouse IgM*	II/41	Rat	14-5790	13-5790		11-5790	
Avidin					18-4100		
Streptavidin*						11-4317	

Secondary reagents listed by format						
Description	Clone	Host	eFluor® 520	eFluor® 570	eFluor® 615	eFluor® 660
F(ab')2 Anti-Mouse IgG*	Polyclonal	Goat		41-4010		50-4010
Anti-Rat IgG1	R1-3G1	Mouse			42-4814	
Anti-Rat IgG2a*	R2a-21B2	Rat		41-4817	42-4817	
Anti-Rat IgG2b	R2b-7C3	Mouse		41-4815	42-4815	
Anti-Mouse IgG1	M1-14D12	Rat		41-4015	42-4015	50-4015
Anti-Mouse IgG2a	m2a-15F8	Rat		41-4210	42-4210	
Anti-Mouse IgM	eB121-15F9	Rat				50-5890
Anti-Mouse IgM	II/41	Rat		41-5790	42-5790	50-5790
Streptavidin*				41-4317	42-4317	50-4317
Phalloidin*			59-6559	41-6559		50-6559

*Representative data can be viewed in this brochure.



ICC using eFluor® 570-conjugated Streptavidin

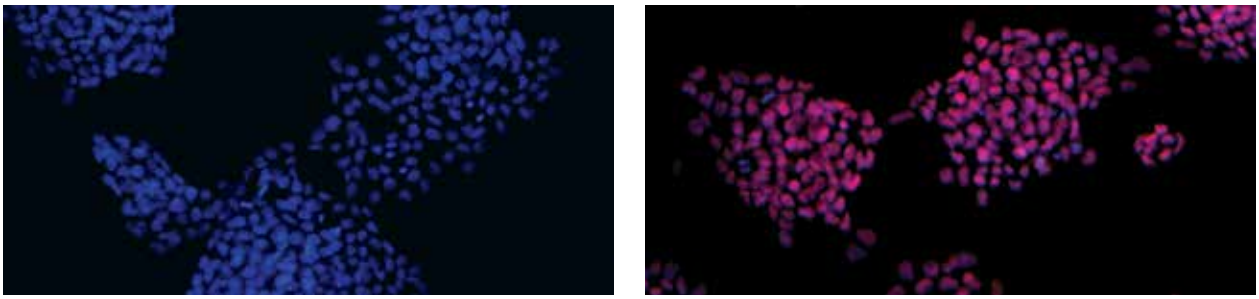
Fixed and permeabilized MDCK cells stained with no primary antibody (left) or 1 ug/mL Anti-alpha Tubulin Biotin (cat. no. 13-4502, right) followed by 1 ug/mL Streptavidin eFluor® 570 (red). Nuclei are stained with DAPI (blue).

Isotype controls

- Confirm specificity of primary antibody binding
- Rule out Fc-receptor–mediated binding and other non-specific interactions

Selecting the appropriate isotype control is an important element in immunostaining experiments. Its purpose is to determine background staining and confirm specificity of the experimental antibody. Isotype controls ideally match the host species, isotype, and conjugation format, in an effort to mimic the non-specific characteristics of the experimental antibodies used.

Isotype controls are developed to assess levels of background staining inherent in cell- and tissue-staining assays. They are a good place to start when optimizing staining protocols (antibody titer, antigen retrieval, secondary reagent titer) using tissue and cells. Staining with an isotype control antibody will help to determine general levels of endogenous fluorescence, or background, in the cells or tissue used, as well as to help identify populations of cells that may have high, non-specific binding as a more relevant negative control.



ICC using eFluor® 570 conjugated isotype-specific secondary antibodies

Fixed and permeabilized F9 murine teratocarcinoma cells stained with Rat IgG2a Isotype Control (left, cat. no. 14-4321) or 5 ug/mL Anti-Human/Mouse Sox2 Purified (right, cat. no. 14-9811), a transcription factor involved in self renewal and pluripotency, followed by 10 ug/mL Anti-Rat IgG2a eFluor® 570 (red). Nuclei are stained with DAPI (blue). Co-expression of Sox2 with DAPI appears pink.

Isotype controls: Purified & Biotin formats				
Description	Clone	Species	Purified	Biotin
Mouse IgG1 K Isotype Control	P3.6.2.8.1	Mouse	14-4714	13-4714
Mouse IgG2a K Isotype Control	eBM2a	Mouse	14-4724	13-4724
Mouse IgG2b K Isotype Control	eBMG2b	Mouse	14-4732	13-4732
Mouse IgG3 Isotype Control*		Mouse	14-4742	
Mouse IgM Isotype Control	11E10	Mouse	14-4752	
Armenian Hamster IgG Isotype Control	299Arm	Armenian Hamster	14-4888	13-4888
Golden Syrian Hamster IgG Isotype Control		Golden Syrian Hamster	14-4914	13-4914
Rat IgG1 K Isotype Control	eBRG1	Rat	14-4301	13-4301
Rat IgG2a K Isotype Control*	eBR2a	Rat	14-4321	13-4321
Rat IgG2b K Isotype Control	eB149/10H5	Rat	14-4031	13-4031

Isotype controls: eFluor® & Organic Dye formats								
Description	Clone	Species	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	Alexa Fluor® 647	eFluor® 660
Mouse IgG1 K Isotype Control	P3.6.2.8.1	Mouse	11-4714	53-4714	41-4714	42-4714	51-4714	50-4714
Mouse IgG2a, K Isotype Control*	eBM2a	Mouse	11-4724	53-4724	41-4724	42-4724	51-4724	50-4724
Mouse IgG2b K Isotype Control*	eBMG2b	Mouse	11-4732	53-4732	41-4732	42-4732	51-4732	50-4732
Mouse IgM Isotype Control	11E10	Mouse	11-4752					50-4752
Mouse IgM Isotype Control	eMM15	Mouse	11-4754					
Armenian Hamster IgG Isotype Control	299Arm	Armenian Hamster	11-4888	53-4888		42-4888	51-4888	50-4888
Golden Syrian Hamster IgG Isotype Control		Golden Syrian Hamster	11-4914	53-4914				
Rat IgG1 K Isotype Control	eBRG1	Rat	11-4301	53-4301	41-4301	42-4301	51-4301	50-4301
Rat IgG2a K Isotype Control	eBR2a	Rat	11-4321	53-4321	41-4321	42-4321	51-4321	50-4321
Rat IgG2b K Isotype Control	eB149/10H5	Rat	11-4031	53-4031	41-4031		51-4031	50-4031

*Representative data can be viewed in this brochure.

Support reagents for IHC and ICC

Buffers and solutions

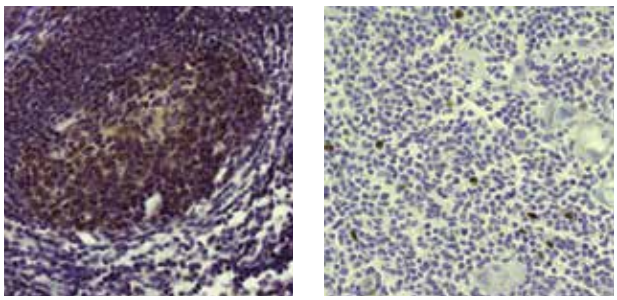
Get the best results possible with support reagents optimized for specific staining protocols, including High and Low Protein Blocking Buffers and slide-mounting media. We offer QC-validated reagents for immunofluorescent staining of tissues and cells with eFluor® conjugated antibodies, in addition to all your routine microscopy applications, such as reducing background staining, minimizing fluorochrome quenching during analysis, and for mounting and long-term storage of slides.

IHC antigen retrieval solutions are designed for use during the heat-induced epitope retrieval (HIER) step prior to immunohistochemistry on formalin-fixed paraffin embedded tissue sections.

- Use in combination with heat (microwave, water bath, or pressure cooker) to restore the antigenicity of proteins modified during the formalin fixation of tissue
- Available in either High pH (10X) or Low pH (10X) formulations

Low and High pH Antigen Retrieval Solutions for optimal signal to noise

Tissue treatment with IHC Antigen Retrieval Solution-Low pH (cat. no. 00-4955) followed by staining with 5 ug/mL Anti-Human AIRE Purified (cat. no. 14-9534) followed by Anti-Rat IgG Biotin (cat. no. 13-4813), Streptavidin HRP, and DAB visualization (left). Tissue treatment with IHC Antigen Retrieval Solution-High pH (cat. no. 00-4956) followed by staining with 5 ug/mL Anti-Human/Mouse Bcl-6 Purified (cat. no. 14-9887) followed by Anti-Mouse IgG Biotin (cat. no. 13-4013), Streptavidin HRP, and DAB visualization (right).



IHC/ICC blocking buffers were developed for use in immunohistochemistry and immunocytochemistry protocols that require blocking of non-specific binding sites. Blocking buffers are recommended for use when staining cells and tissues to block nonspecific antibody binding. Use in blocking step, and as a diluent for eFluor® dye-conjugated antibodies.

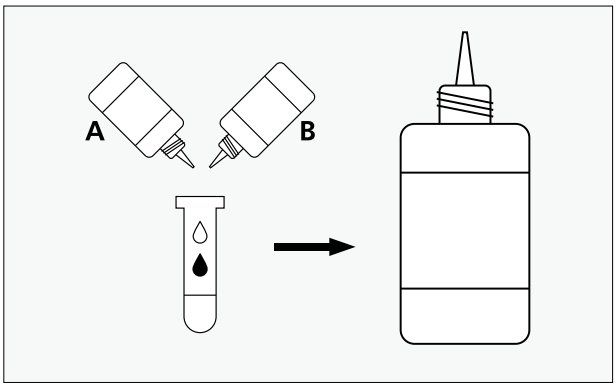
- Two formulations available; compatible with organic dye-conjugated antibodies or unconjugated antibodies for 2- and 3-step staining protocols
- Low protein formulation is ideal for applications using unconjugated or organic dye-conjugated antibodies
- High protein formulation provides optimal results when staining nuclear antigens, for FFPE tissue sections, or tissues with higher non-specific background

Fluoromount-G™ and Fluoromount-G™ with DAPI is a clear liquid medium designed for use in mounting slides following immunofluorescent staining. This water-soluble medium is used to mount slides in which the final step of staining is aqueous. It forms a semi-permanent seal for prolonged storage of slides at 2-8°C.

- Does not fluoresce and may reduce the amount of fluorochrome quenching during fluorescence microscopy
- Compatible with eFluor® organic dye-conjugated antibodies, as well as other dyes
- Convenient, ready-to-use 1X solution
- Available with DAPI for nuclear visualization

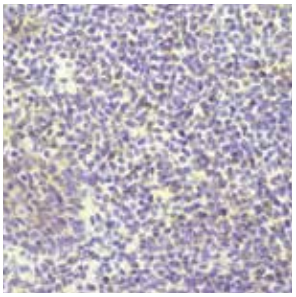
DAB Advanced Chromogenic Kit contains the necessary reagents to prepare a DAB substrate working solution. The enzyme peroxidase (Horseradish Peroxidase/HRP) uses the DAB substrate (3, 3'-diaminobenzidine tetrahydrochloride) producing a brownish-red precipitate. These precipitates are insoluble in organic solvent. This enzyme/substrate reaction can be used on tissue sections or cells (grown on culture slides or cytospins) to visualize the localization of an HRP-conjugated antibody in immunocytochemistry and immunohistochemistry applications. DAB is used in IHC as a secondary staining reagent, characterized by a brown reaction. Many labs reconstitute DAB from a powder, although it is a suspected carcinogen, so less handling is better.

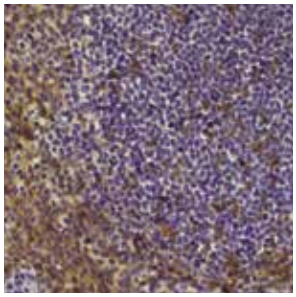
Preparation of DAB reagent



Simple to use

- Solution-based format
- Minimal handling
- Mix reagents A and B
- Easy dropper bottle application



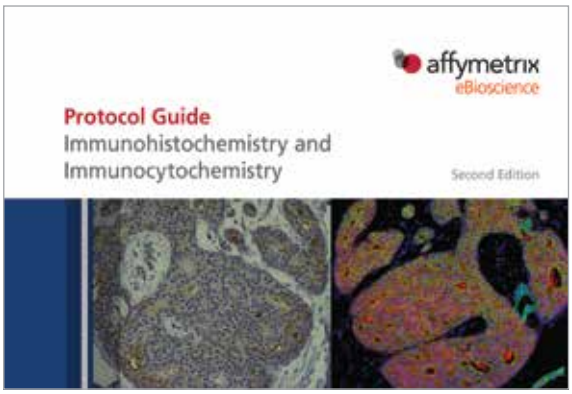


Improved results - achieve greater sensitivity

Comparison of Anti-Human CD4 Purified (cat. no. 14-0045) staining of FFPE human tonsil tissue with homemade DAB (left) and DAB Advanced Chromogenic Kit (right, cat. no. 8801-4965).

Support products		
Description	Product	Cat. No.
Calcein AM Viability Dye (UltraPure Grade)	Dye	65-0853
Calcein Blue AM Viability Dye	Dye	65-0853
CalceinViolet 450 AM Viability Dye	Dye	65-0854
Calcium Sensor Dye eFluor® 514	Dye	65-0840
Indo-1 AM Calcium Sensor Dye	Dye	65-0856
Indo-1 AM Calcium Sensor Dye (UltraPure Grade)	Dye	65-0857
CellVue® Burgundy Cell Labeling Kit	Dye	88-0872
CellVue® Lavender Cell Labeling Kit	Dye	88-0873
CellVue® Maroon Cell Labeling Kit	Dye	88-0870
CellVue® Plum Cell Labeling Kit	Dye	88-0871
CellVue® NIR815 Cell Labeling Kit	Dye	88-0874
CellVue® NIR780 Cell Labeling Kit	Dye	88-0875
CellVue® Jade Cell Labeling Kit	Dye	88-0876
DRAQ5™*	Dye	65-0880
CyTRAK Orange™	Dye	65-0881
Cell Proliferation Dye eFluor® 670	Dye	65-0840
CFSE	Dye	65-0850
Fura-2 AM Dye	Dye	65-0858
JC-1 Mitochondrial Membrane Potential Dye	Dye	65-0851
IHC/ICC Blocking Buffer - High Protein	Buffer/Solution	00-4952
IHC /ICC Blocking Buffer - Low Protein	Buffer/Solution	00-4953
20X TBS Wash Buffer for IHC/ICC	Buffer/Solution	00-4954
CellVue Diluent C	Buffer/Solution	00-4501
IHC Antigen Retrieval Solution – Low pH (10X)*	Buffer/Solution	00-4955
IHC Antigen Retrieval Solution – High pH (10X)*	Buffer/Solution	00-4956
DAB Advanced Chromogenic Kit*	Kit	8801-4965
Fluoromount-G™	Buffer/Solution	00-4958
Fluoromount G™ with DAPI	Buffer/Solution	00-4959
Apo-Direct Apoptosis Detection	Kit	88-6671
Apo-BrdU Apoptosis Detection	Kit	88-6671
StainTray™	Accessory	44-0404

*Representative data can be viewed in this brochure.



Ask an eBioscience representative how to obtain the IHC/ICC Protocol Guide

- Direct and indirect protocols
- Colorimetric and immunofluorescent staining techniques
- At-a-glance workflow diagrams
- Required materials lists
- Troubleshooting guide

Reference tools

Fluorophore compatibility table for multicolor experiments

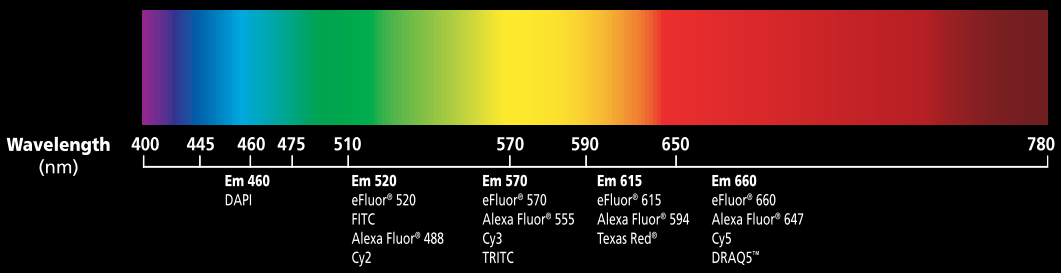
	DAPI	eFluor® 520 FITC Alexa Fluor® 488 Cy2 GFP	eFluor® 570 Alexa Fluor® 555 Cy3 TRITC	eFluor® 615 Alexa Fluor® 594 Texas Red®	eFluor® 660 Alexa Fluor® 647 Cy5 DRAQ5™
DAPI		Yes	Yes	Yes	Yes
eFluor® 520, FITC, Alexa Fluor® 488, Cy2, GFP	Yes		Yes	Yes	Yes
eFluor® 570, Alexa Fluor® 555, Cy3, TRITC	Yes	Yes		No	Yes
eFluor® 615, Alexa Fluor® 594, Texas Red®	Yes	Yes	No		Possible with spectral imaging
eFluor® 660, Alexa Fluor® 647, Cy5, DRAQ5™	Yes	Yes	Yes	Possible with spectral imaging	

Yes - indicates emission spectra of the two fluorophores can be distinguished using typical/standard filter sets for fluorophores listed (see below).
No - indicates using typical/standard filter sets for the fluorophore listed will not result in adequate discrimination of the two emission spectra. In some cases, using an adapted or optimized filter set or spectral imaging may allow discrimination of overlapping or adjacent emission spectra of fluorophores.

Standard filter sets for fluorophores

Dye	Excitation (nm)	Dichroic	Emission (nm)
DAPI	365/50	400LP	450/65
eFluor® 520, FITC, Alexa Fluor® 488, Cy2, GFP	475/40	510LP	535/45
eFluor® 570, Alexa Fluor® 555, Cy3, TRITC	546/12	570LP	585/40
eFluor® 615, Alexa Fluor® 594, Texas Red®	560/55	585LP	645/75
eFluor® 660, Alexa Fluor® 647, Cy5, DRAQ5™	620/60	660LP	700/75

Immunofluorescence—fluorophores for multicolor microscopy
eFluor® direct conjugates named according to peak emission



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