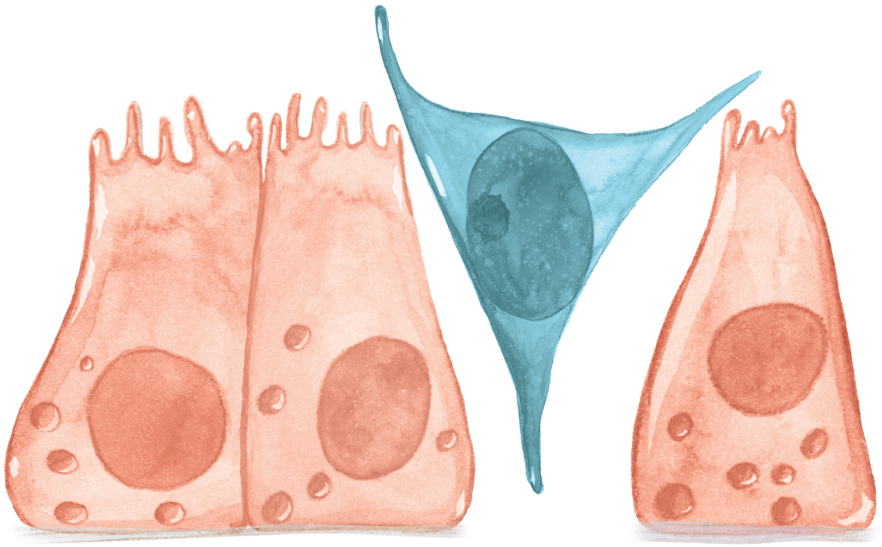
R&D Systems Tools for Cell Biology Research™

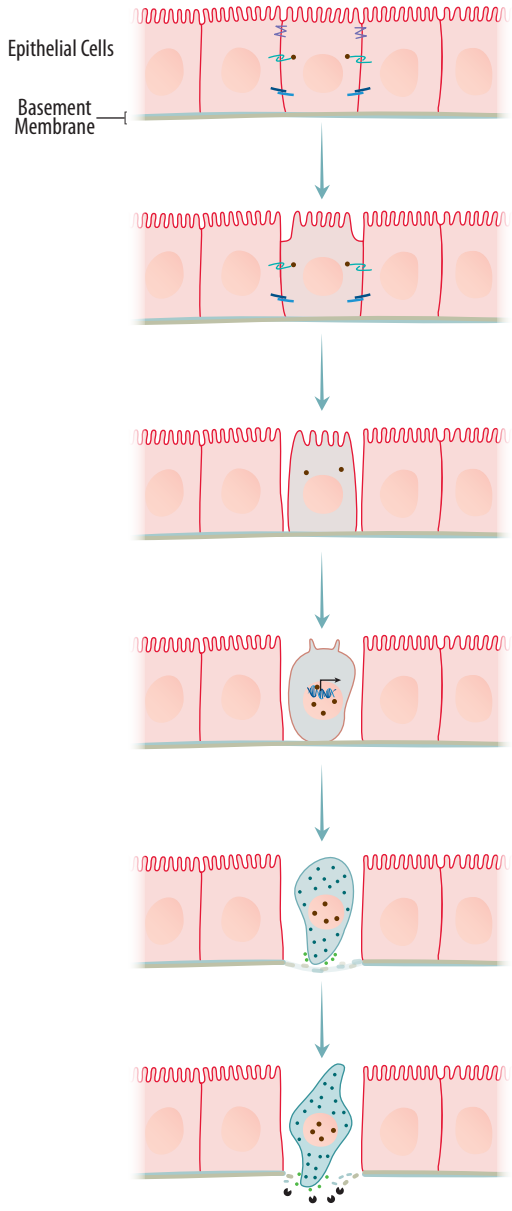
Epithelial to Mesenchymal Transition



Three Distinct Forms of Epithelial to Mesenchymal Transition

Epithelial to Mesenchymal Transition (EMT) describes a mechanism by which cells lose their epithelial characteristics and acquire more migratory mesenchymal properties. This transient and reversible process can be classified into three subtypes, depending on the biological and functional setting in which it occurs. Type 1 EMT underlies the generation of secondary epithelia and is critical during embryogenesis and organ development. The formation of fibroblasts during wound healing and fibrosis is considered a second distinct EMT subtype, while type 3 EMT is characterized by the transformation of epithelial cells into the invasive metastatic mesenchymal cells that underlie cancer progression.

The Progressive Stages of EMT



Epithelial Layers

Epithelial cells compose highly organized layers that make up functionally diverse sheets, tubes, and vesicles. Individual cells display apical-basal polarity and are closely adjoined by tight junctions (≳), adherens junctions (⌇), and desmosomes (▬). Adherens junctions are anchored to the cytoskeleton by β-Catenin (•).

Key Molecules (EMT Effectors): EGF, FGF acidic, HGF, PDGF, TGF-β, Wnt-3a

Loss of Tight Junctions

One of the earliest events during EMT involves disassembly of the lateral intercellular seals close to the apical surface. This triggers redistribution of key molecules, disruption of the polarity complex, and cytoskeletal reorganization.

Key Molecules: Actin, Claudins, JAM, Occludin, p120, Par3, Par6, Zona Occludens

Loss of Adherens Junctions & Desmosomes

Adherens junctions composed of E-Cadherin, and desmosomes made up of a desmoglein and a desmocollin are dismantled. The molecules that anchor these specialized cell-cell contacts to the cytoskeleton are redistributed.

Key Molecules: α-Actinin, α-Catenin, β-Catenin, E-Cadherin, Desmocollins, Desmogleins, Plakoglobin, Plakophilin, Vinculin

Cytoskeletal Changes

Following loss of junctional complexes and downregulation of E-Cadherin, β-Catenin is no longer sequestered in the cytoplasm and translocates to the nucleus to activate β-Catenin responsive genes. The actin cytoskeleton forms stress fibers that anchor to focal adhesion complexes and promote cell migration.

Key Molecules: Actin, Cytokeratins, S100A4, α-Smooth Muscle Actin, Vimentin

Transcriptional Shift

Snail, ZEB, and bHLH family transcription factors suppress epithelial markers and activate mesenchymal genes. The cytoskeletal protein vimentin is upregulated (•), as is the deposition of the extracellular matrix (ECM) protein Fibronectin (•).

Key Molecules: Ets-1, FOXC2, Goosecoid, LEF-1, Snail 1, Snail 2 (Slug), Twist-1, ZEB1, ZEB2

Increased Motility & Migration

ECM components stimulate integrin signaling and promote the formation of focal adhesion complexes. Upregulation of N-Cadherin increases cell motility. Metalloproteinases (◕) degrade the extracellular matrix and facilitate cell migration.

Key Molecules: N-Cadherin, FAK, Fibronectin, α5β6 Integrin, Laminin-5, SPARC, Syndecan-1, Vitronectin

Type 1 - Developmental

EMT generates mesenchymal cells which undergo mesenchymal to epithelial transition (MET) to form secondary epithelia. This is essential for gastrulation, neural crest cell migration, and organ development.

Type 2 - Wound Healing

EMT generates fibroblasts in response to tissue injury. Failure to cease following attenuation of inflammation causes organ fibrosis. The kidneys, liver, lungs, and intestine are particularly vulnerable.

Type 3 - Cancer Metastasis

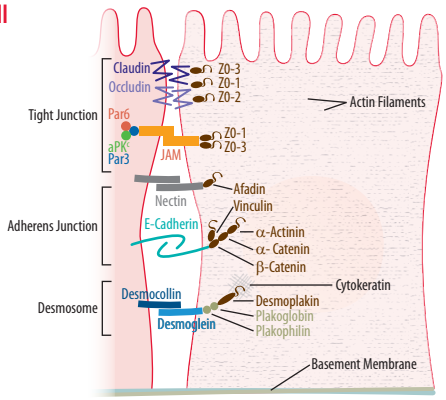
EMT allows neoplasic cells to become motile and invasive, and leave the primary epithelial tumor site. A distal MET event promotes the formation of a secondary tumor and cancer progression in other organs.

This illustration represents general pathways suggested in the scientific literature and is not to be considered comprehensive nor definitive.

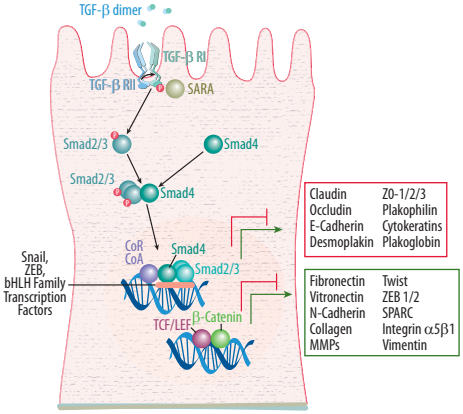
TGF-β as an EMT Effector

Many studies have supported the importance of TGF-β signaling for the repression of epithelial genes and induction of mesenchymal genes *in vitro* and *in vivo*. Activation of the TGF-β signaling pathway requires a heteromeric receptor complex composed of two type I and two type II transmembrane serine-threonine kinase receptors. Following binding of TGF-β, type II receptors phosphorylate type I receptors, which in turn phosphorylate Smad2 and Smad3. Smad2/3 forms a trimer with Smad4 which translocates to the nucleus and interacts with transcription factors, co-activators, and co-repressors. In addition, cross-talk between TGF-β and PDGF, Wnt, and Notch signaling pathways has been shown to contribute to EMT.

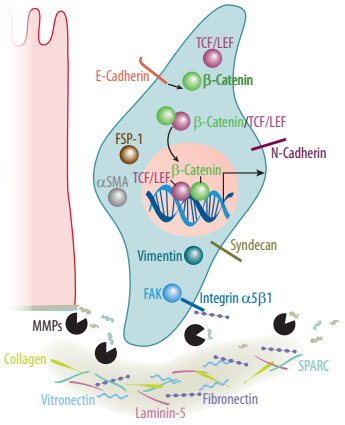
Polarized Epithelial Cell



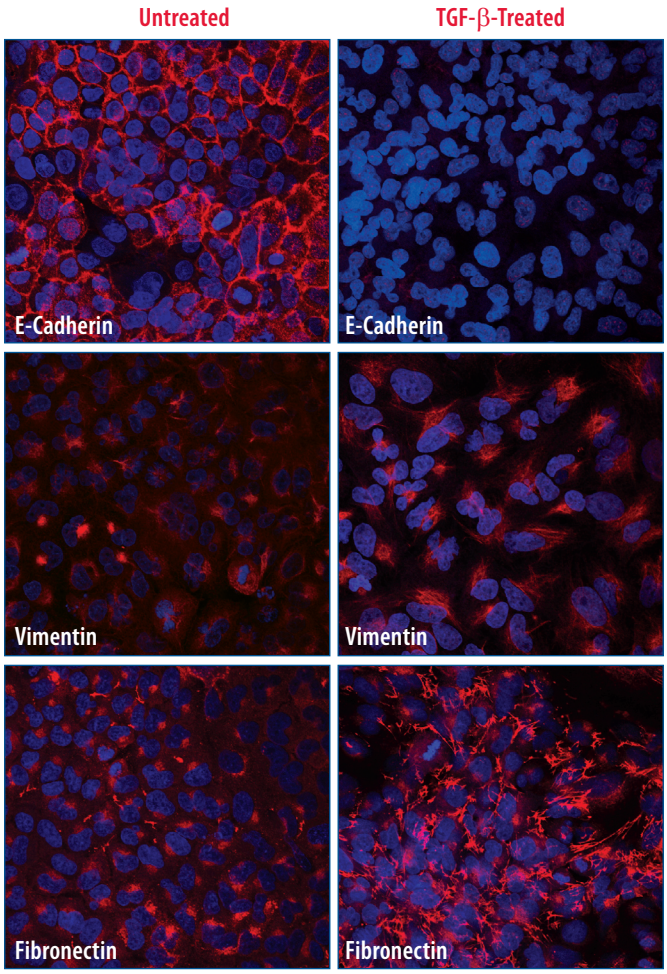
TGF-β-induced EMT



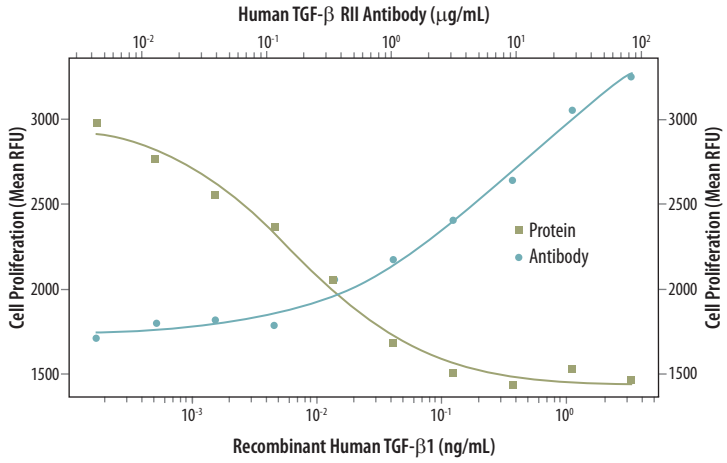
Migratory Mesenchymal Cell



TGF-β Signaling Induces EMT. TGF-β signaling causes the repression of epithelial genes and induces the expression of mesenchymal markers. During this process polarized epithelial cells become migratory mesenchymal cells.



Induction of EMT by TGF-β. A549 human lung carcinoma cells were treated with 10 ng/mL Recombinant Human Transforming Growth Factor-β (TGF-β) (Catalog # 240-B) for 24 hours to induce EMT. E-Cadherin, Vimentin, and Fibronectin were detected using Human E-Cadherin (Catalog # AF648), Human Vimentin (Catalog # AF2105), and Human Fibronectin (Catalog # AF1918) Antigen Affinity-purified Polyclonal Antibodies. Cells were stained for E-Cadherin and Vimentin using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001, red) and for Fibronectin using the NorthernLights 557-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL010, red). Nuclei were counterstained using DAPI (blue). Following TGF-β treatment, ICC studies revealed downregulation of the epithelial marker E-Cadherin and concurrent upregulation of the mesenchymal markers Vimentin and Fibronectin.



Neutralization of TGF-β by Human TGF-β Receptor II Antibody. Recombinant Human Interleukin-4 (rhIL-4, Catalog # 204-IL) induces cell proliferation in TF-1 human erythroleukemic cell line. Addition of Recombinant Human Transforming Growth Factor-β1 (rhTGF-β1, Catalog # 100-B) inhibits IL-4-induced proliferation in a concentration dependent manner (green line). The ED_{50} for this effect is typically 0.005 - 0.02 ng/mL in the presence of 5 ng/mL rhIL-4. The inhibition by rhTGF-β1 was neutralized (blue line) by increasing concentrations of Human TGF-β1 Receptor II Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-241-NA). The Neutralization Dose (ND_{50}) for this antibody was determined to be approximately 10 - 20 μg/mL.

R&D Systems Products for EMT Research

For more information, please visit our website at
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Epithelial Markers

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
Actin		H M R	
ALCAM	H M	H M	H M
β-Catenin		H M R X	H
E-Cadherin	H M	H M	H M
Claudin-1, -3, -4		H	
Collagen I	R B		
Cytokeratin 8		H	
Cytokeratin 14		H	
Cytokeratin 19		H	
Desmocollin-1	H		
Desmocollin-2	H	H	
Desmocollin-3	H	H	
Desmoglein-1	H	H	
Desmoglein-2	H	H	
Desmoglein-3	H	H	
EpCAM	H	H	H
Hyaluronan*			Ms
JAM-A	H M	H M	M
JAM-B/VE-JAM	H M	H M	
JAM-C	H M	H M	
JAM-4/IGSF5		M	
Laminin I	M		
Nectin-1	H	H	
Nectin-2/CD112	H M	H	
Nectin-3	H	H M	
Nectin-4		H M	

Mesenchymal Markers

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
α-Smooth Muscle Actin		H	
N-Cadherin	H	H	
DDR2	H	H	
Desmin		H	
FAK	H	H M R	H M R
Fibronectin	H B	H	
Integrin αV/CD51		H	
Integrin β1/CD29		H M	
Laminin-5		H	
MMP-2	H M R	H M R	H
MMP-3	H M	H M	H M
MMP-9	H M R	H M	H M
S100A4	H M	H M	
Syndecan-1/CD138	H M	H M	
Vimentin	H	H	
Vitronectin	H B	H M	

EMT Effectors

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
BMP-7	H M	H	H
EGF	H M R	H M R	H M R
FGF acidic	H M B	H B	H
HGF	H M Ca	H M Ca	H M
PDGF	H M R P	H M R Ms	H M R
TGF-β	H M A P	H M Ms	H M R Ca P
Wnt-3a	H M	M	

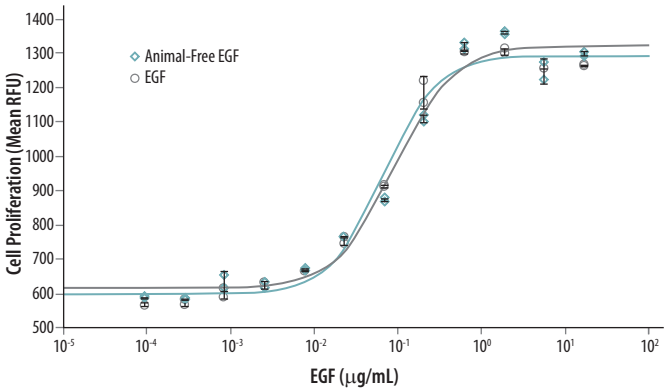
EMT Signaling Molecules

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
Akt		H M R	H M R
Dishevelled-1		H	
Dishevelled-2		H	
Dishevelled-3		H	
Dkk-1	H M R	H M R	H M
Dkk-2	M	M	
Dkk-3	H	H M	H
Dkk-4	H M	H M	H
EOMES		H	
FoxC2		H	
Goosecoid		H	
GSK-3β		H M R	H M R
HMGA2		H M	
HNF-4α/NR2A1		H	
ICAT		H	
ILK		H M R	
Jagged 1	H R	H R	
Jagged 2	H M	H	
JNK		H M R	H M R
Noggin	H M	M	
Notch 1, 2, 3, 4	H M R	H M R	
p38 MAP Kinase		H M R	H M
p300		H	
Rap1A/B		H M R	
Ras		H M R	
Smad2		H M	H
Smad2/3		H M	H
Smad3		H M	H
Smad7		H M R	
Snail		H	H
Sonic Hedgehog	H M	H M	M
Src	H V	H M R	H
STAT3		H M R	H M
Twist-1		H	

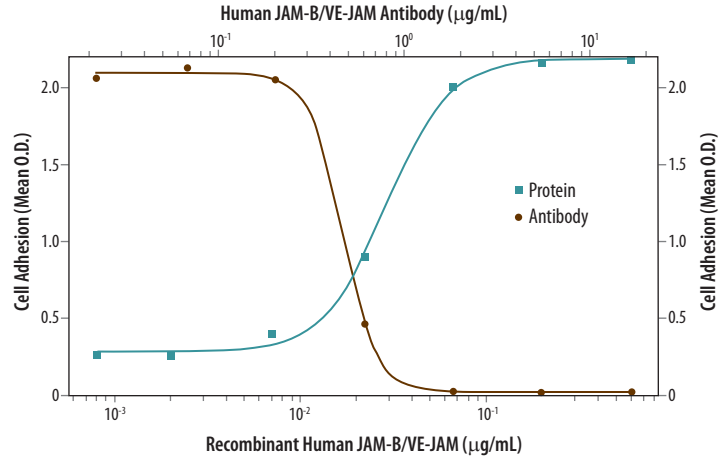
* Available as Ultralow, Low, Medium, and High molecular weight polymers.

KEY: **H:** Human **M:** Mouse **R:** Rat **A:** Amphibian **B:** Bovine **Ca:** Canine **Ms:** Multispecies **P:** Porcine **V:** Viral **X:** *Xenopus*

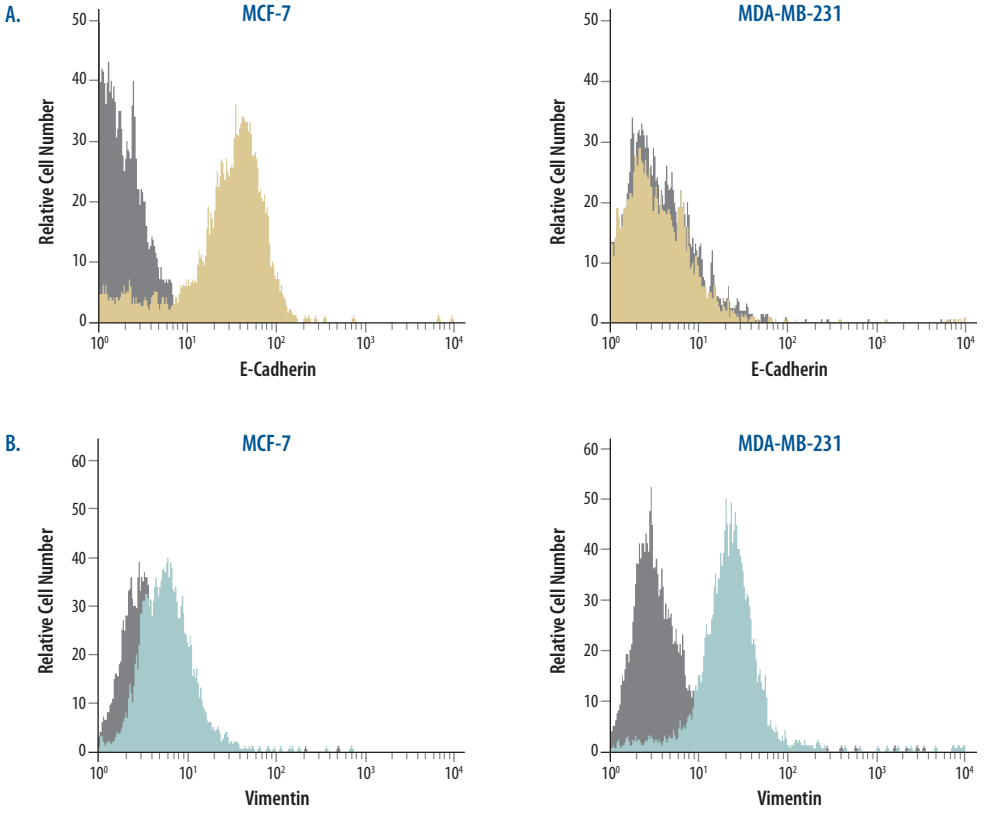
R&D Systems Products for EMT Research



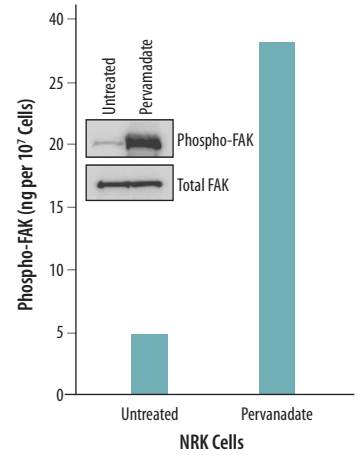
Bioactivity of Animal-Free Derived EGF. Balb/3T3 mouse embryonic fibroblast cell line proliferation was studied following treatment with the indicated concentrations of Animal-Free™ Recombinant Human Epidermal Growth Factor (EGF, Catalog # AFL236, blue line) or Recombinant Human EGF produced in a standard laboratory environment (Catalog # 236-EG, gray line). Cell number was assessed by a fluorometric assay using the redox sensitive dye, Resazurin (Catalog # AR002). These data confirm the high bioactivity of our animal-free product and demonstrates that it is comparable to the same molecule produced under typical conditions. EGF has been shown to induce EMT in a variety of cell lines and is believed to promote the metastasis of several different forms of cancer.



JAM-B-induced Cell Adhesion and Neutralization by Human JAM-B Antibody. Recombinant Human Junction-associated Molecule B (rhJAM-B, Catalog # 1074-VJ) immobilized onto a microplate previously coated with goat anti-human Fc, increases cell adhesion in J45.01 human acute lymphoblastic leukemia T lymphocyte cell line in a concentration dependent manner (blue line). At the end of the incubation, non-adherent cells were washed off, and cells attached to the wells were detected by measuring endogenous cellular lysosomal acid phosphatase activity. Cell adhesion elicited by 0.2 µg/mL rhJAM-B is neutralized (brown line) by increasing concentrations of Human JAM-B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074). The Neutralization Dose (ND_{50}) for this antibody was determined to be approximately 0.2 – 0.8 µg/mL.



Detection of E-Cadherin and Vimentin in MCF-7 and MDA-MB-231 Cells by Flow Cytometry. A. MCF-7 and MDA-MB-231 human breast cancer cell lines were labeled with Phycoerythrin (PE)-conjugated Human E-Cadherin Monoclonal Antibody (Catalog # FAB18381P, tan histogram) or PE-conjugated Mouse IgG_{2B} Isotype Control Antibody (Catalog # IC0041P, gray histogram). **B.** MCF-7 and MDA-MB-231 human breast cancer cell lines were labeled with PE-conjugated Human Vimentin Monoclonal Antibody (Catalog # IC2105P, blue histogram) or PE-conjugated Mouse IgG_{2A} Isotype Control Antibody (Catalog # IC0041P, gray histogram). MCF-7 cells, which are considered non-metastatic, express high levels of the epithelial marker E-Cadherin and low levels of the mesenchymal marker Vimentin. The opposite expression profile was observed in MDA-MB-231 cells, which are considered to be metastatic breast cancer cells.



Detection of Phospho-FAK by ELISA and Western Blot. Focal Adhesion Kinase 1 (FAK) phosphorylated at Y397 was detected in lysates of NRK rat normal kidney cell line using the Rat Phospho-FAK (Y397) DuoSet® IC ELISA (Catalog # DYC4528). Cells were untreated or treated with 100 µM sodium pervanadate for 10 minutes to induce FAK phosphorylation. For comparison the same lysates were also immunoblotted (inset) with either Human/Mouse/Rat Phospho-FAK (Y397) (Catalog # AF4528) or Rat FAK Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4467). FAK is a non-receptor protein tyrosine kinase that is essential for cell attachment and movement.

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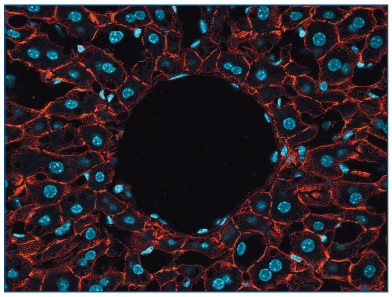
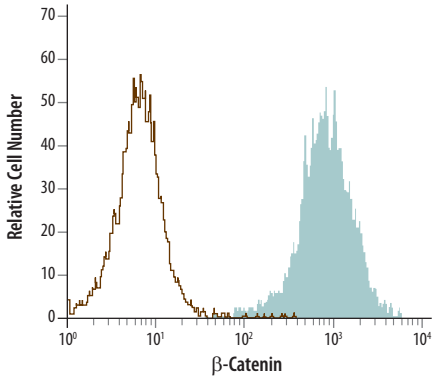
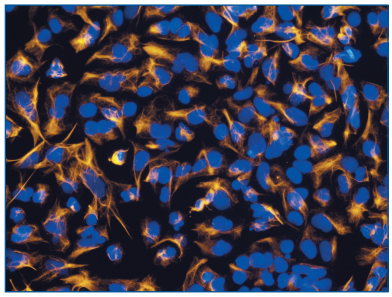
Antibodies for Epithelial & Mesenchymal Markers



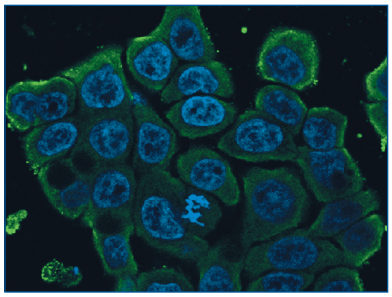
ALCAM in Mouse Liver. Activated Leukocyte Cell Adhesion Molecule (ALCAM) was detected in frozen sections of mouse liver using Mouse ALCAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172). Tissue was stained using the NorthernLights 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001, red) and nuclei were counterstained with DAPI (blue). Specific labeling was confined to the plasma membrane of hepatocytes surrounding the central vein.



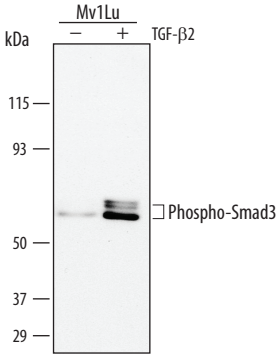
Intracellular Detection of β-Catenin in Human HeLa Cells by Flow Cytometry. HeLa human cervical epithelial carcinoma cells were labeled with Allophycocyanin-conjugated Human β-Catenin Monoclonal Antibody (Catalog # IC13292A, blue histogram) or Allophycocyanin-conjugated Mouse IgG_{2A} Isotype Control Antibody (Catalog # IC003A, brown histogram). During EMT, β-Catenin translocates from the cytoplasm to the nucleus to activate β-Catenin responsive mesenchymal genes.



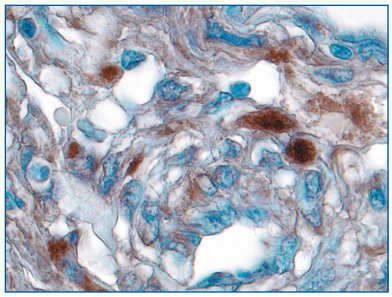
Vimentin in Human NTera-2 Cells. Vimentin was detected in NTera-2 human testicular embryonic carcinoma cell line using Human Vimetin Monoclonal Antibody (Catalog # MAB2105). Cells were stained using the NorthernLights 557-conjugated Anti-Rat IgG Secondary Antibody (Catalog # NL013, yellow) and nuclei were counterstained with DAPI (blue). Vimentin belongs to the intermediate filament family, it is highly expressed in mesenchymal cells, and has been used as a specific mesenchymal marker.



EpCAM in Human HT-29 Cells. Epithelial Cellular Adhesion Molecule (EpCAM) was detected in HT-29 human colon adenocarcinoma cell line using Human EpCAM Monoclonal Antibody (Catalog # MAB960). Cells were stained using the NorthernLights 493-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # NL009, green) and nuclei were counterstained with DAPI (blue). EpCAM expression is increased in actively proliferating epithelia tissues and in human malignant neoplasms.



Detection of Phospho-Smad3 by Western Blot. Western blot shows lysates of Mv1Lu mink lung epithelial cell line untreated (-) or treated (+) with 10 ng/mL Recombinant Human Transforming Growth Factor-β2 (TGF-β2, Catalog # 302-B2) for 24 hours. PVDF membrane was probed with Human Phospho-Smad3 (S423/S425) Antigen Affinity-purified Polyclonal Antibody (Catalog # AB3226), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008).



Jagged-1 in Human Kidney Cancer Tissue. Jagged-1 was detected in paraffin-embedded sections of human kidney cancer tissue using Human Jagged-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1277). Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS008, brown) and counterstained with hematoxylin (blue). Jagged-1 activates Notch signaling pathways, which have been shown to contribute to both physiological and pathological forms of EMT.