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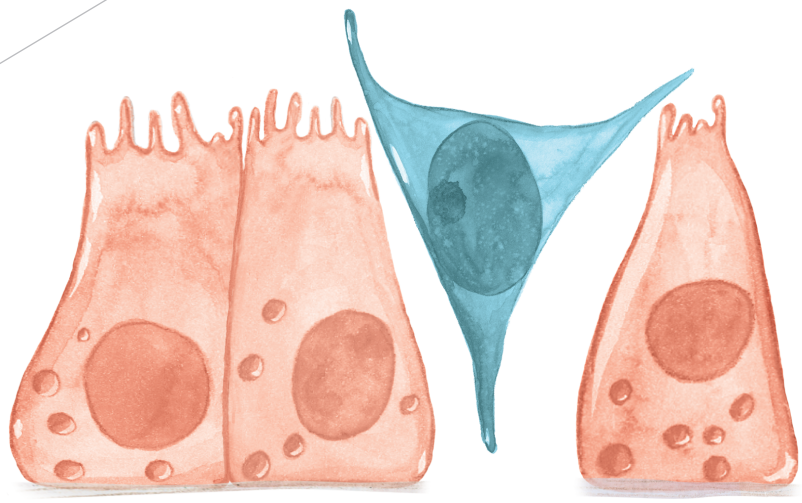
Epithelial to Mesenchymal Transition



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 is classified into three subtypes that are dependent on the biological and functional setting in which it occurs.

Type 1 - Developmental

EMT during development is essential for gastrulation, neural crest cell migration, and organ development.

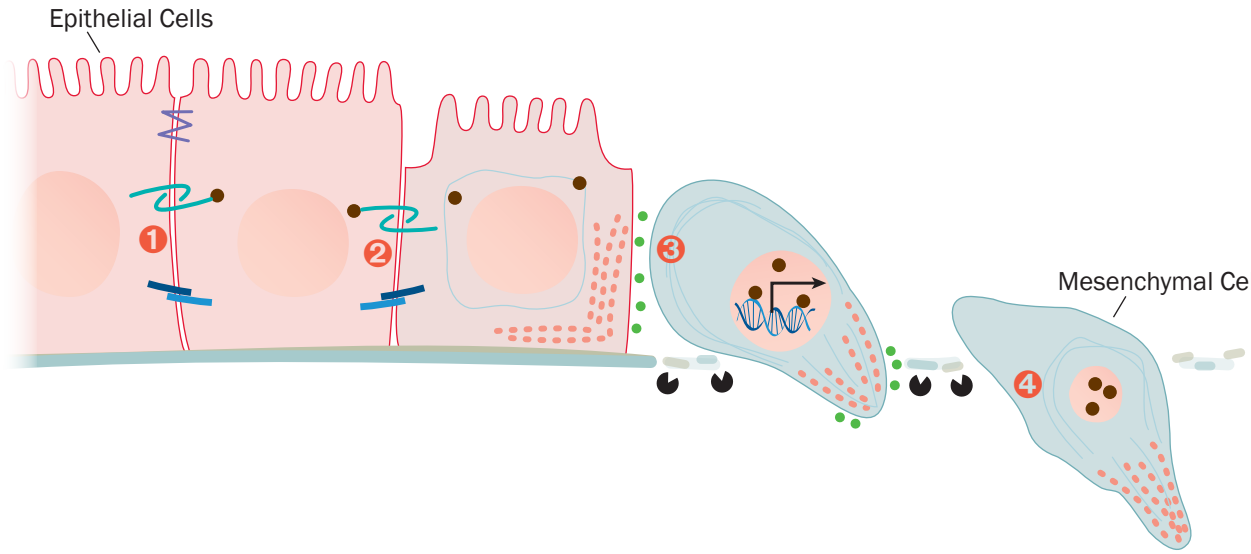
Type 2 - Wound Healing

EMT generates fibroblasts following tissue injury that assist in local wound healing. Persistent EMT following attenuation of inflammation can result in organ fibrosis.

Type 3 - Cancer Metastasis

EMT results in the transformation of epithelial cells into the invasive metastatic mesenchymal cells that underlie cancer progression.

The Progressive Stages of EMT



1 Loss of Tight Junctions, Adherens Junctions, and Desmosomes

- Disassembly of specialized cell-cell contacts leads to redistribution of cytoskeletal proteins and disruption of the apical-basal cell polarity of epithelial cells.
- **Key Molecules:** Actin, α-Actinin, α-Catenin, β-Catenin, Claudins, E-Cadherin, Desmogleins, Desmocollin, JAM, Occludin, Plakoglobin, Plakophilin, Vinculin, Zona Occludens

2 Cytoskeletal Changes

- Formation of actin stress fibers that anchor to focal adhesion complexes to begin to promote cell migration.
- **Key Molecules:** Actin, Cytokeratins, S100A4, α-Smooth Muscle Actin, Vimentin

3 Transcriptional Shift

- Suppression of epithelial genes and activation of mesenchymal genes is mediated by Snail, ZEB, and bHLH family transcription factors. Vimentin is upregulated and extracellular deposition of Fibronectin is increased.
- **Key Molecules:** FoxC2, Goosecoid, LEF-1, Snail 1, Snail 2 (Slug), Twist-1, ZEB1, ZEB2

4 Increased Migration and Motility

- Upregulation of N-Cadherin, secretion of matrix metalloproteases, and stimulation of integrins by extracellular matrix proteins facilitates cell motility.
- **Key Molecules:** N-Cadherin, FAK, Fibronectin, α5β6 Integrin, Laminin-5, SPARC, Syndecan-1, Vitronectin

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

Products for EMT Research

Epithelial Markers

Molecule	Recombinant and Natural Proteins	Antibodies	ELISAs
ALCAM/CD166	H M	H M R Ca	H M
Amnionless	H	H M	
Claudin-1, -3, -4, -6		H	
HNF-3β		H	
Cytokeratin 8, 14, 18, 19		H	
E-Cadherin	H M R	H M	H M
EpCAM/TROP-1	H	H	H
Hyaluronan*			Ms
IGSF4C/SynCAM4	H	H	
JAM-4/IGSF5		M	
JAM-A	H M	H M	M
JAM-B/VE-JAM	H M	H M	
JAM-C	H M	H M	
Laminin-1		M	
MSP R/Ron	H M	H M	H
Nectin-1	H	H M	
Nectin-2/CD112	H M	H M	
Nectin-3	H	H M	
Nectin-4	H M	H M	H
Occludin		H	
Desmocollin-1	H		
Desmocollin-2, -3	H	H	
Desmoglein-1, -2, -3	H	H	

Mesenchymal Markers

Molecule	Recombinant and Natural Proteins	Antibodies	ELISAs
α-Smooth Muscle Actin		H	
Cadherin-11	H M	H	
Cyr61/CCN1	H	H M	H
DDR2	H M	H	H
Desmin		H M	
FAK	H	H M R	H M R
Fibronectin	H B	H	H
Integrin α1/CD49a		H	
Integrin β1/CD29		H M P Ca	
L1CAM	H M	H M	
Laminin α3/Laminin-5		H	
MMP-2	H M R	H M R	H M R P Ca
MMP-3	H M	H M	H M
MMP-9	H M R	H M	H M R
N-Cadherin	H M	H M R	
S100A4	H M	H M	
SPARC	H M	H M	H
Syndecan-1/CD138	H M	H M	H
Tenascin C	H	H M	
Vimentin	H	H M	
Vitronectin	H B	H M	

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

EMT Signaling Molecules

Molecule	Recombinant and Natural Proteins	Antibodies	ELISAs
Akt		H M R	H M R
Cortactin		H R	
ALK-1	H M	H M	H M
DDR1	H M	H	H
Dishevelled-1, -2, -3		H	
Dkk-1	H M R	H M	H M
Dkk-2	H M	M	
Dkk-3	H	H M	H
ERK1, 2	H	H M R	H M R
Fibulin 5/DANCE	H	H	
FoxC2		H M	
Goosecoid		H	
GSK-3β	H	H M R	H M R
ILK		H M R	
Jagged 1	H R	H M R	H R
Jagged 2	H M	H M	
JNK		H M R	H M R
KLF4, 5, 10, 17		H	
MFG-E8	H M	H M	H M
MUC-1, -4, -19		H	
NEDD9/CASL		H	
NFκB1		H M	
Nidogen-1/Entactin	H	H	H
Noggin	H M	M	
Notch-1	H M R	H M R	H
Notch-3	H M	H M	
p300		H	
p38		H M R	H M
PINCH1		H M R	
Rap1A/B		H M R	
Ras		H	
SHP-2	H	H M R	H M R
Slug		H	
Smad2		H M D	
Smad3		H M	
Smad7		H M R	
SMURF2		H M R	
Snail		H	H
Sonic Hedgehog/Shh	H M	H M	M
SPRED2		H	
Src	H V	H M R	H
TAZ/WWTR1		H	
Twist-1		H	
Versican		H	
WIF-1	H M	H M	H
YY1		H M	
ZEB 1		H	

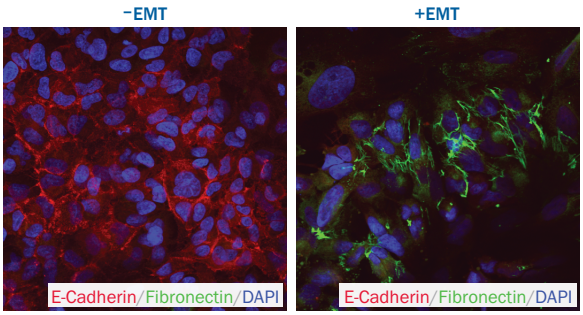
Species Key: **H** Human **M** Mouse **R** Rat **B** Bovine **Ca** Canine **D** *Drosophila* **Ms** Multiple Species **P** Porcine

EMT Induction and Verification Kits

StemXVivo™ EMT Inducing Media Supplement

Drives EMT in Cells Resistant to TGF-β

- **Rapid** - induces EMT in only 5 days
- **Versatile** - compatible with multiple cell types
- **Consistent** - defined formulation results in reproducible EMT induction



Induction of EMT with StemXVivo EMT Inducing Media Supplement. A549 human lung carcinoma cell cultures were either untreated (–EMT) or treated (+EMT) with media containing the StemXVivo EMT Inducing Media Supplement (Catalog # CCM017) for 5 days. EMT induction resulted in reduced E-Cadherin expression (red) and increased Fibronectin labeling (green). E-Cadherin was detected in cells using a NorthernLights™ (NL) 577-Conjugated Goat Anti-Human E-Cadherin Antigen Affinity-Purified Polyclonal Antibody (Catalog # NL648R). Fibronectin was detected using a Mouse Anti-Human Fibronectin Monoclonal Antibody (Catalog # MAB1918) followed by a NL493-Conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL009). The nuclei were counterstained with DAPI (blue).

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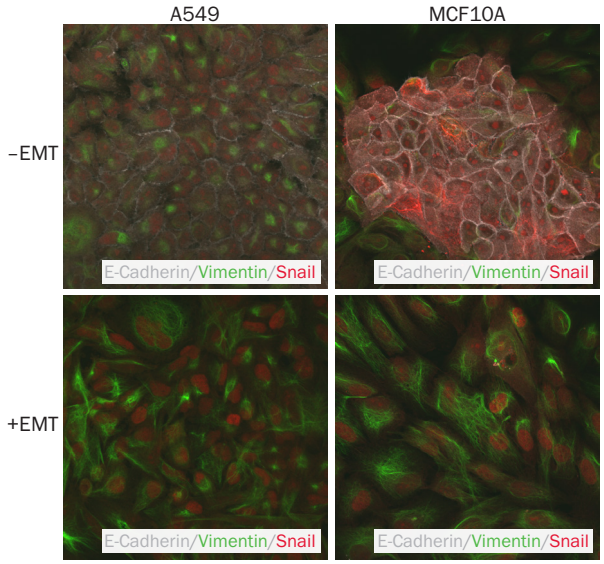
Human EMT 3-Color Immunohistochemistry Kit

An EMT Research Essential

- **Thorough** – determines EMT status by protein expression level and subcellular localization
- **Efficient** – single-step staining using fluorescently-labeled primary antibodies
- **Time-Saving** – screens for multiple markers simultaneously



Confirmation of EMT Using the Human EMT 3-Color Immunocytochemistry Kit. A549 human lung carcinoma and MCF10A human breast epithelial cell cultures were either untreated (–EMT) or treated (+EMT) with media containing the StemXVivo EMT Inducing Media Supplement (Catalog # CCM017). The cells were analyzed for EMT using the antibodies included in the EMT 3-Color Immunocytochemistry Kit (Catalog # SC026). Compared to untreated cells, cells cultured in EMT Inducing Media downregulated the epithelial marker, E-Cadherin (pseudocolored white), and upregulated the mesenchymal markers, Vimentin (green) and Snail (red).

Cutting-Edge Research from R&D Systems

EMT induction and verification kits are featured in the Journal of Visualized Experiments (JoVE).

Induction and Analysis of Epithelial to Mesenchymal Transistion. In this article, the R&D Systems research team demonstrates a straightforward method for the induction of EMT in a variety of cell types. Methods for analyzing cells pre- and post-EMT induction are highlighted, including immunocytochemical staining, antibody-based array analysis, and migration/invasion assays. *Tang, Y. et al. (2013) J. Vis. Exp.* ***78****:e50478.*

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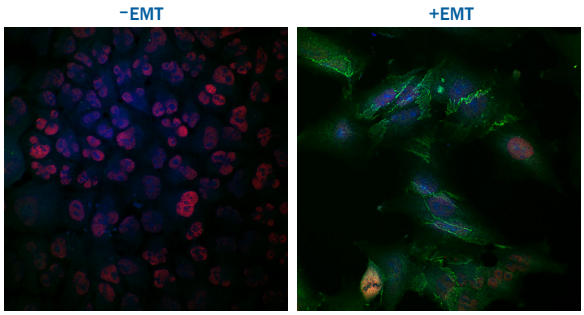
Essential Antibodies to Characterize EMT Status

Markers to Monitor EMT

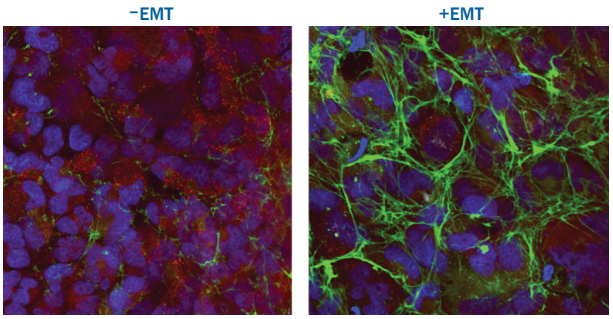
HNF-3β and Cadherin-11



HNF-3β and Cadherin-11 Expression During EMT. The A549 human lung carcinoma cell line was incubated with untreated media (-EMT) or with media containing the StemXVivo EMT Inducing Media Supplement (+EMT; Catalog # CCM017) for 5 days. Cells were stained for the transcription factor HNF-3β/FoxA2 (Catalog # AF2400) and the mesenchymal cell marker Cadherin-11 (Catalog # MAB1790) followed by the NorthernLights™ (NL)557-Conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001) and NL493-Conjugated Anti-Mouse IgG Secondary Antibody (Catalog # NL009), respectively. HNF-3β (red) is expressed in untreated A549 cells and decreased in EMT-induced cells. Conversely, Cadherin-11 (green) expression is high in EMT-induced cells and not in untreated cells. The nuclei were counterstained with DAPI (blue).

Upregulated During EMT

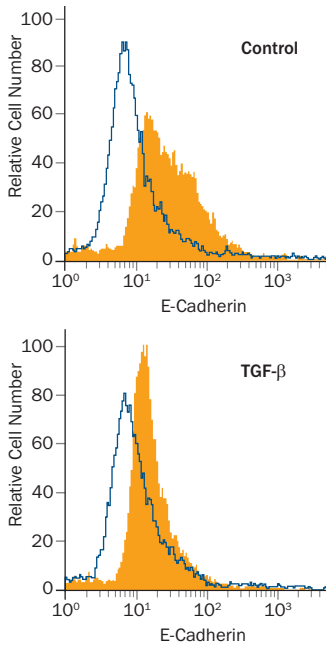
Fibronectin



Detection of Fibronectin in EMT-Induced Cells. Fibronectin is upregulated in T98G glioblastoma cells induced into EMT (+EMT) with media containing the StemXVivo EMT Inducing Media Supplement (Catalog # CCM017) compared to untreated cells (–EMT). Fibronectin was detected using the Mouse Anti-Human Fibronectin Monoclonal Antibody (green; Catalog # MAB1918) followed by the NorthernLights™ (NL)493-Conjugated Goat Anti-Mouse Secondary Antibody (Catalog # NL009). The cells were counterstained for E-Cadherin (red) and DAPI (blue). *From Tang, Y. et al. (2013) J. Vis. Exp.* ***78****:e50478.*

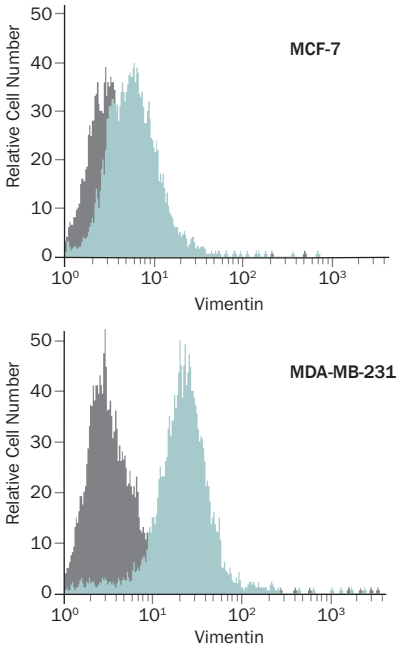
Quantify EMT Using Flow Cytometry

E-Cadherin



Reduced E-Cadherin Expression Following TGF-β-Induced EMT. EMT was induced in the A549 human lung carcinoma cell line with cell culture media supplemented with Recombinant Human (rh)TGF-β1 (Catalog # 240-B). Control cells were cultured without rhTGF-β1. EMT induction was confirmed at 48 h by flow cytometric staining with the PE-conjugated Mouse Anti-Human E-Cadherin Monoclonal Antibody (filled; Catalog # FAB18381P), an epithelial cell marker, or a PE-conjugated Mouse IgG2B Isotype Control Antibody (open; Catalog # IC0041P). TGF-β1 decreased the expression of E-Cadherin.

Vimentin



Vimentin Expression is Upregulated in Metastatic Breast Cancer Cells. The metastatic human breast cancer cell line, MDA-MB-231, and the non-metastatic human breast cancer cell line, MCF-7, were labeled for the mesenchymal cell marker, Vimentin. Cells were stained with Rat Anti-Human Vimentin PE-Conjugated Monoclonal Antibody (blue histogram; Catalog #IC2105) or the Mouse IgG_{2A} Isotype Control Antibody (gray histogram). Expression of Vimentin was higher in MDA-MB-231 cells compared to non-metastatic MCF-7 cells.

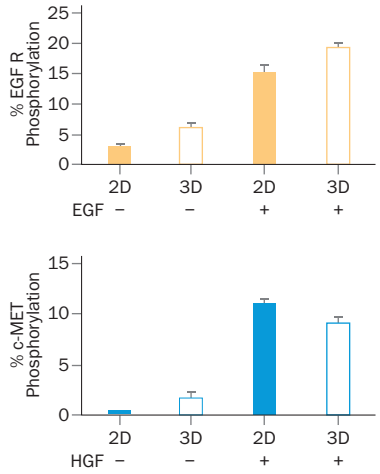
Products to Investigate EMT

EMT Effector Proteins: Highest Purity on the Market

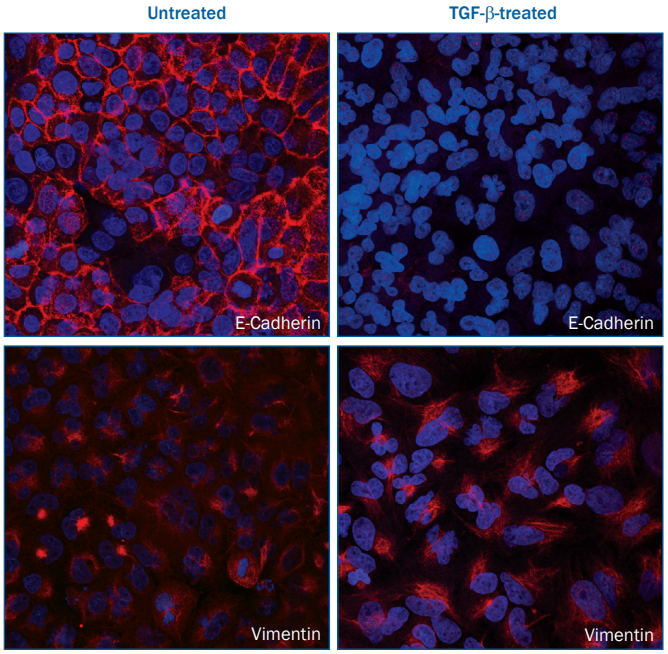
Recombinant Proteins

- **Consistent Performance** – each lot is tested for consistency to ensure that culture conditions remain the same across experiments
- **Guaranteed Bioactivity** – rigorously tested for high bioactivity using relevant cell culture systems
- **World-Class Purity** – all proteins meet our industry-leading endotoxin specifications (<0.1 EU/μg)

	Catalog #	
Molecules	**Human**	**Mouse**
BMP-7	354-BP	5666-BP
EGF	236-EG	2028-EG
FGF acidic	232-FA	4686-FA
HGF	294-HG	2207-HG
IL-6	206-IL	406-ML
Notch-1	3647-TK	5627-TK
PDGF-BB	220-BB	
TGF-β1	240-B	7666-MB
Wnt-3a	5036-WN	1324-WN
Wnt-3a High Purity	5036-WNP	1324-WNP



Recombinant Human EGF and HGF Stimulation Increase EGF R and c-MET Phosphorylation in 3D Lung Tumor Spheroids. Day four monolayer (**2D**) and spheroid (**3D**) A549 lung carcinoma cell cultures were stimulated with (+) or without (–) 100 ng/ml of Recombinant Human (rh)EGF (Catalog # 236-EG) or rhHGF (Catalog # 294-HG) for 15 minutes. Cell lysates from rhEGF-stimulated and rhHGF-stimulated cultures were collected and analyzed for phosphorylation of EGF R and c-MET, respectively. N is equal to 4 replicates per condition. *Data were adapted from Ekert, J.E. et al. (2014) PLoS One* **9***:e99248.*



Induction of EMT by TGF-β. A549 human lung carcinoma cells were cultured in control media (Untreated) or media supplemented with Recombinant Human (rh) TGF-β1 (TGF-β-treated; Catalog # 240-B). rhTGF-β1 treatment resulted in the downregulation of the epithelial marker E-Cadherin (red) and concurrent upregulation of the mesenchymal marker Vimentin (red). Cells in separate wells were stained with either a Goat Anti-Human E-Cadherin (Catalog # AF648) or Goat Anti-Human Vimentin (Catalog # AF2105) Antigen Affinity-Purified Polyclonal Antibody. The NorthernLights™ (NL)557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001) was used to visualize E-Cadherin and Vimentin. Nuclei were counterstained using DAPI (blue).

Functional Assays for EMT: Publication-Ready

Cell Invasion and Migration Assays

- **Quantitative** – measures cell movement through extracellular matrices
- **Flexible** – different basement membrane extract (BME) densities and ECM proteins are available
- **Published** – assays are featured in many peer reviewed journals

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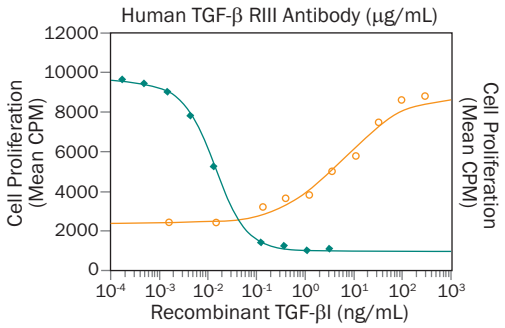
Product		**Catalog #**
CultreCoat® Invasion Assay	BME Optimization Assay	3484-096-K
	Low BME	3481-096-K
	Medium BME	3482-096-K
	High BME	3483-096-K
Cultrex® Invasion Assay	BME	3455-096-K
	Laminin I	3456-096-K
	Collagen I	3457-096-K
	Collagen IV	3458-096-K
Cultrex® 96 Well Cell Migration Assay		3465-096-K

Modulators of EMT: Confirmed Bioactivity

Neutralizing Antibodies

Antibody	Catalog #
Human TGF-β Receptor II Affinity Purified Polyclonal Ab	AF-241-NA
Human EGF Polyclonal Ab	AB-236-NA
Human HGF R/c-MET Affinity Purified Polyclonal Ab	AF276
Human HGF Polyclonal Ab	AB-294-NA
Human IL-6 Rα Affinity Purified Polyclonal Ab	AF-227-NA
Human IL-6 Affinity Purified Polyclonal Ab	AF-206-NA
Human/Mouse Wnt-3a MAb (Clone 217804)	MAB1324
Human PDGF Rα Affinity Purified Polyclonal Ab	AF-307-NA
Human BMP-7 MAb (Clone 164311)	MAB3541



Neutralization of TGF-β by Human TGF-β Receptor II Antibody. Addition of Recombinant Human TGF-β1 (rhTGF-β1, Catalog # 240-B) inhibits Recombinant Human IL-4 (rhIL-4)-induced proliferation of TF-1 human erythroleukemic cell line in a concentration dependent manner (green line). The inhibition by rhTGF-β1 was neutralized (orange line) by increasing concentrations of Human TGF-β1 RII Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF-241-NA) with a ND_{50} of 5–20 µg/mL.

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EMT-Related Small Molecules from Tocris Bioscience

Extracellular Matrix

Product Name	Catalog #	Product Description
Batimastat	2961	Potent, broad spectrum MMP inhibitor
BIO 5192	5051	Highly potent and selective inhibitor of integrin α4β1
L-685,458	2627	Potent and selective γ-secretase inhibitor

Growth Factor and Associated Receptors

Product Name	Catalog #	Product Description
BMS 536924	4774	Dual IR/IGF1R inhibitor
BMS 599626 dihydrochloride	5022	Potent, selective EGFR and ErbB2 inhibitor
GSK 1838705	5111	Potent and selective IR and IGF1R inhibitor; antitumor
Iressa	3000	Orally active, selective EGFR inhibitor
PD 173074	3044	FGFR1 and -3 inhibitor
PHA 665752	2693	Potent and selective MET inhibitor
SB 431542	1614	Potent, selective inhibitor of TGF-βRI, ALK4 and ALK7
SD 208	3269	Potent ATP-competitive TGF-βRI inhibitor
SU 5402	3300	Potent FGFR and VEGFR inhibitor
Sunitinib malate	3768	Potent VEGFR, PDGFRβ and KIT inhibitor

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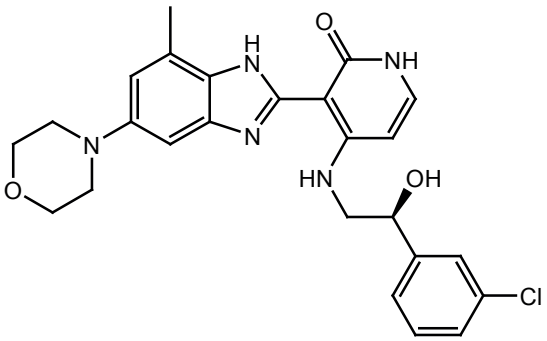

BMS 536924 - Catalog # 4774
BMS 536924 is a dual inhibitor of the insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF1R) (IC_{50} values are 73 and 100 nM respectively). In IGF1R overexpressing MCF10A cells, this compound reverses EMT through the attenuation of *Snail* mRNA expression and the restoration of E-cadherin protein expression. BMS 536924 also inhibits cell proliferation in multiple tumor types.

Signaling Pathways

Product Name	Catalog #	Product Description
Dynasore	2897	Non-competitive dynamin inhibitor
EHT 1864	3872	Potent inhibitor of Rac family GTPases
Garcinol	4827	PCAF/p300 HAT inhibitor; anticancer
GSK 2830371	5140	Potent and selective allosteric inhibitor of Wip1 phosphatase
ICG 001	4505	Inhibits TCF/β-catenin-mediated transcription
IPA 3	3622	Group I p21-activated kinase (PAK) inhibitor
IWP 2	3533	PORCN inhibitor; inhibits Wnt processing and secretion
NSC 23766	2161	Selective inhibitor of Rac1-GEF interaction; antioncogenic
PD 0325901	4192	Potent inhibitor of MEK1/2
Y-27632 dihydrochloride	1254	Selective p160ROCK inhibitor

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