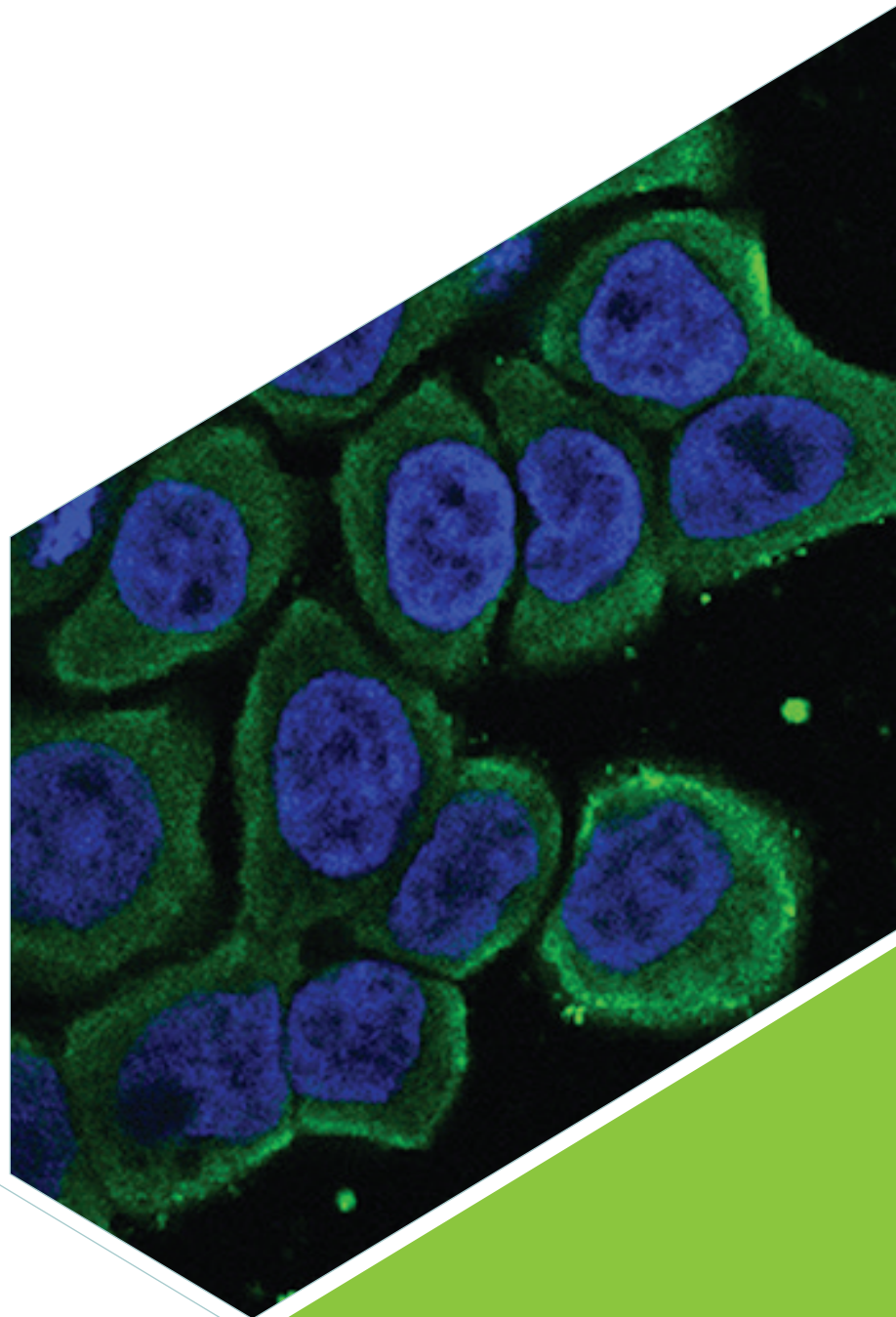


Cancer Stem Cells

ISOLATE & CULTURE

VERIFY

INVESTIGATE

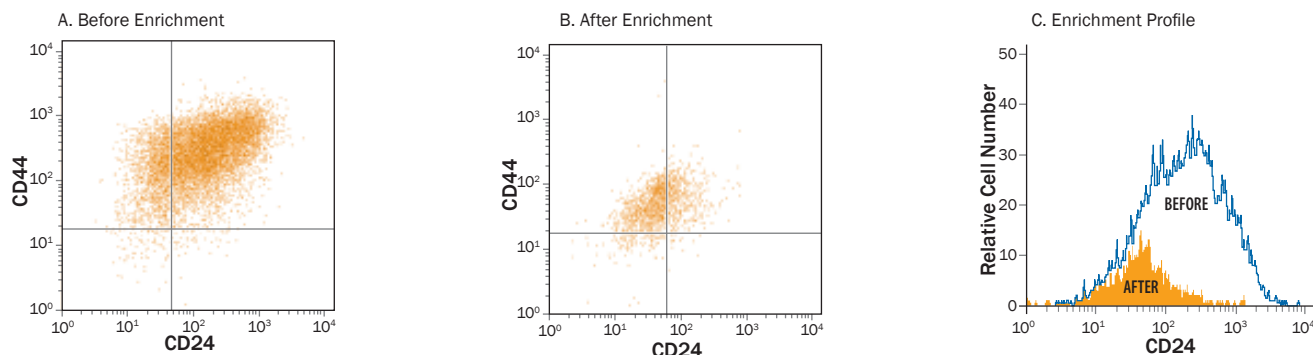


ISOLATE AND CULTURE

MagCollect™ Cell Selection Kits for Cancer Stem Cell Research

- **Fast**—target cells can be enriched in approximately 1 hour
- **Flexible**—compatible with several magnet systems
- **Specific**—negative and positive selection with two antibodies improves purity of recovered cells
- **Safe**—No cell damage induced by beads or ferrofluids

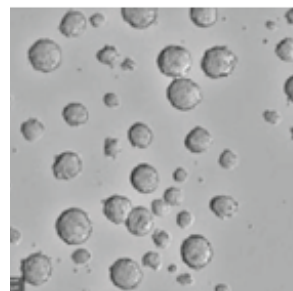
MagCollect™ Kit	Description	Catalog #
CD24 ^{low} -CD44 ⁺ Breast Cancer Stem Cell Isolation Kit	Isolates CD24 ^{low} -CD44 ⁺ breast cancer stem cells	MAGH111
Human EpCAM ⁺ Cell Isolation Kit	Isolates Epithelial Cell Adhesion Molecule expressing cells via positive selection	MAGH121
Mouse Mesenchymal Stem Cell Isolation Kit	Isolates MSCs via negative selection	MAGM212B



Analysis of CD24^{low}-CD44⁺ Cells Isolated using the MagCollect™ Human CD24^{low}-CD44⁺ Breast Cancer Stem Cell Isolation Kit. A population of CD24^{low}-CD44⁺ cells was isolated from the MCF-7 human breast cancer cell line using the MagCollect™ CD24^{low}-CD44⁺ Breast Cancer Stem Cell Isolation Kit (Catalog # MAGH111). CD24^{low}-CD44⁺ cells (upper left quadrant), before (A) and after (B) enrichment, were detected by double-staining with APC-conjugated Mouse Anti-Human CD24 and PE-conjugated Mouse Anti-Human CD44 Detection Antibodies (both provided in the kit). A histogram profiling the enrichment of CD24^{low}- cells (filled histogram) from the original cell population (open histogram) is also shown (C).

StemXVivo® Serum-Free Tumorsphere Media

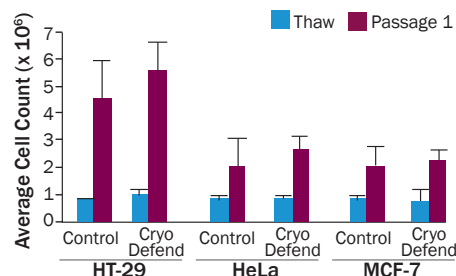
- Specialized semi-solid media optimized for tumorsphere formation
- Shown to support sphere formation of seven tumor cell lines
- Lot-to-lot consistency reduces experimental variability
- Specially formulated and optimized for tumorsphere formation



Tumorsphere Formation in MCF-7 Human Breast Cancer Cells. StemXVivo® Serum-Free Tumorsphere Media was used to form tumorspheres in MCF-7 human breast cancer cells. The cells were plated at 3×10^4 cells per well in a 6-well ultralow adhesion culture plate and cultured at 37 °C and 5% CO₂ for 3–10 days.

CryoDefend®-Cell Lines Cryopreservation Media

- Protein-free cryopreservation media
- Fully-defined to reduce experimental variability
- Supports post-thaw cell viability better than conventional freezing media
- Suitable for stem cells and cancer cell lines



Cryopreservation in CryoDefend®-Cell Lines Media. Cancer cell lines cryopreserved (1×10^6 cells/cryovial) in either control freezing media (90% culture media/10% DMSO) or CryoDefend®-Cell Lines (CryoDefend) media were thawed, counted (blue bars), resuspended in standard culture media, and plated into T75 flasks. After 3–5 days in culture, the cells were harvested and counted (red bars). Error bars shown indicate the standard deviation.

Antibodies for Cancer Stem Cell Markers

Molecule	Species
Bladder Cancer Stem Cell Markers	
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
CD44	H M R Ca
CD47	H M
CEACAM-6/CD66c	H
Breast Cancer Stem Cell Markers	
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
BMI-1	H
CD24	H
CD44	H M R Ca
Connexin 43/GJA1	H M R B Ca Ch Pr Z Fi GP S X
CXCR1/IL-8 RA	H
CXCR4	H M F
DLL4	H M
EpCAM/TROP1	H
ErbB2/Her2	H M
GLI-1	H M
GLI-2	H M
IL-1 α /IL-1F1	H M R CR P
IL-6 R α	H M
Integrin α 6/CD49f	H M B
PON1	H M
PTEN	H M R
Colon Cancer Stem Cell Markers	
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
ALCAM	H M R
CD44	H M R Ca
CD133	H
DPPIV/CD26	H M
EpCAM/TROP1	H
GLI-1	H M
Lgr5/GPR49	H M
Musashi-1	H
Gastric Cancer Stem Cell Markers	
CD44	H M R Ca
DLL4	H M
Lgr5/GPR49	H M
Glioma/Medulloblastoma Cancer Stem Cell Markers	
A20/TNFAIP3	H
ABCG2	H
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
BMI-1	H
CD15/Lewis X	H
CD44	H M R Ca
CD133	H
CX3CL1/Fractalkine	H M R

Molecule	Species
CX3CR1	H M
CXCR4	H M F
HIF-2 α /EPAS1	H M R
IL-6 R α	H M
Integrin α 6/CD49f	H M B
L1CAM	H M
Musashi-1	H
c-Myc	H
Nestin	H M R
Podoplanin	H M
SOX2	H M
Head & Neck Cancer Stem Cell Markers	
ABCG2	H
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
BMI-1	H
CD44	H M R Ca
HGF R/c-MET	H M Ca
Lgr5/GPR49	H M
Leukemia Stem Cell Markers	
BMI-1	H
CD34	H M R Ca P
CD38	H M
CD44	H M R Ca
CD47	H M
CD96	H M
CD117/c-kit	H M
GLI-1	H M
GLI-2	H M
IL-3 R α /CD123	H M
MICL/CLEC12A	H M
Musashi-2	H
TIM-3	H M
Liver Cancer Stem Cell Markers	
α -Fetoprotein	H M
Aminopeptidase N/ANPEP	H M
CD45	H M
CD90/Thy1	H M
CD117	H M
EpCAM	H M
NF2/Merlin	H
Lung Cancer Stem Cell Markers	
ABCG2	H
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
CD90/Thy1	H M
CD117/c-kit	H M
EpCAM/TROP1	H

Molecule	Species
Melanoma Stem Cell Markers	
ABCB5	H
ABCG2	H
ALCAM	H M R
CD133	H
MS4A1/CD20	H
Nestin	H M R
NGF R/TNFRSF16	H M
Myeloma Stem Cell Markers	
ABCB5	H
CD19	H M R
CD27/TNFRSF7	H M
CD38	H M
MS4A1/CD20	H
Syndecan-1	H M
Osteosarcoma Cancer Stem Cell Markers	
ABCG2	H
CD44	H M R Ca
Endoglin/CD105	H M
Nestin	H M R
STRO-1	H
Ovarian Cancer Stem Cell Markers	
α -Methylacyl-CoA Racemase/AMACR	H
CD44	H M R Ca
CD117/c-kit	H M
Endoglin/CD105	H M
Ovastacin	H
Pancreatic Cancer Stem Cell Markers	
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
BMI-1	H
CD24	H
CD44	H M R Ca
CXCR4	H M F
EpCAM/TROP1	H
PON1	H M
Prostate Cancer Stem Cell Markers	
α -Methylacyl-CoA Racemase/AMACR	H
ABCG2	H
ALCAM	H M R
Aldehyde Dehydrogenase 1-A1/ALDH1A1	H
BMI-1	H
CD44	H M R Ca
CD151	H
c-MAF	H
c-Myc	H
TRA-1-60(R)	H

Species Key:

H Human
M Mouse

R Rat
B Bovine

Ca Canine
Ch Chicken

CM Cynomolgus
Macaque

CR Cotton Rat
F Feline

Fi Finch
P Porcine

Pr Primate
S Sheep

X Xenopus
Z Zebrafish

INVESTIGATE

Target Cancer Stem Cell Signaling Pathways

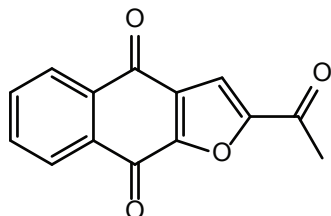
Tocris® Products for Cancer Stem Cell (CSC) Research

According to the CSC hypothesis, CSCs must be eradicated to eliminate a tumor and prevent its recurrence. Tocris Bioscience offers products to inhibit key enzymes and signaling pathways that are utilized by CSCs and are currently being investigated as a potential new field of cancer therapeutics^{1,2,3}. Additionally, the Tocriscreen™ Stem Cell Toolbox, a new compound library collection, is now available from Tocris. The Tocriscreen™ Stem Cell Toolbox is ideal for both high-throughput and high content screening, providing an indispensable starting point for modern drug discovery. Visit www.tocris.com to explore the variety of other molecules and screening compound libraries on offer.

References

1. Takebe, N. *et al.* (2011) *Nat. Rev. Clin. Oncol.* **8**:97.
2. Wang, K. *et al.* (2013) *Int. J. Nanomedicine* **8**:899.
3. Li, Y. *et al.* (2015) *Proc. Natl. Acad. Sci. USA.* **112**:1839.

Product Name	Description	Catalog #
Screening Library		
Tocriscreen™ Stem Cell Toolbox	80 chemical modulators for Stem Cell Research supplied as pre-dissolved DMSO solutions (250 µl 10 mM solution per compound)	5060
Wnt Pathway Inhibitors		
ICG 001	Inhibits TCF/β-catenin-mediated transcription	4505
IWP 2	PORCN inhibitor; inhibits Wnt processing and secretion	3533
endo-IWR 1	Wnt/β-catenin signaling inhibitor; axin stabilizer	3532
Hedgehog Pathway Inhibitors		
Ciliobrevin A	Hedgehog (Hh) pathway antagonist, inhibits ciliogenesis	4529
Cyclopamine	Smoothed inhibitor	1623
HPI 1	Inhibits Shh-, SAG-, and Gli-induced Hedgehog pathway activation in Shh-LIGHT2 cells	3839
Notch Pathway Inhibitors		
DAPT	γ-secretase inhibitor	2634
DBZ	γ-secretase inhibitor	4489
L-685,458	γ-secretase inhibitor	2627
Other Key Enzyme Inhibitors		
BRD 7116	Inhibitor of leukemia stem cell activity	5336
Geldanamycin	Breast cancer stem cell inhibitor; selective Hsp90 inhibitor	1368
ITE	Induces stem-like cancer cell differentiation; also inhibits TGF-β-induced human myofibroblast differentiation	1803
ML 239	Breast cancer stem cell inhibitor	4829
Napabucasin	Blocks cancer stem cell self-renewal; STAT3 inhibitor	5522
Niclosamide	Inhibits AML stem cells; STAT3 and mTORC1 signaling inhibitor	4079
STF 118804	Depletes leukemia stem cells; NAMPT inhibitor	5207
Thioridazine hydrochloride	Selective inducer of CSC differentiation; anticancer agent	3070
TY 52156	Inhibits S1P-induced breast cancer stem cell expansion; S1P ₃ receptor antagonist	5328
Verteporfin	Suppresses cancer stem cell properties; YAP inhibitor	5305



Napabucasin (Catalog # 5522)

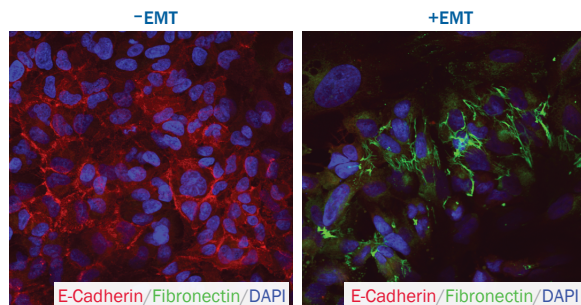
Napabucasin is a STAT3 inhibitor that blocks spherogenesis of cancer stem cells (CSC), but not hematopoietic stem cells. It also inhibits PaCa-2 xenograft tumor growth in mice, even after cessation of treatment, and decreases population of CSCs in PaCa-2 tumors from treated mice. Napabucasin also blocks spleen and liver metastases of colon cancer cells in a mouse model.

INVESTIGATE

Epithelial to Mesenchymal Transition (EMT)

StemXVivo® EMT Inducing Media Supplement

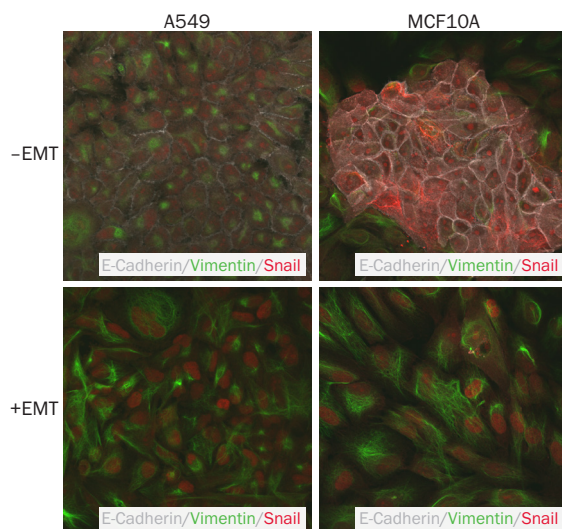
- **Inclusive**—drives EMT in cells resistant to TGF- β
- **Rapid**—induces EMT in only 5 days
- **Versatile**—compatible with multiple cell types
- **Consistent**—defined formulation results in reproducible EMT induction



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).

Human EMT 3-Color Immunocytochemistry Kit

- **Essential**—includes antibodies to identify epithelial and mesenchymal cells
- **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).

INVESTIGATE

Cancer Cell Behavior

Cell Migration & Invasion Assays

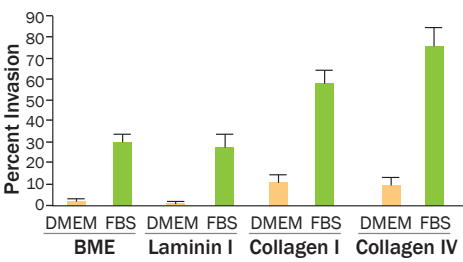
Cultrex® Cell Migration & Invasion Assays	Catalog #
BME Cell Invasion Assay	3455-096-K
Laminin I Cell Invasion Assay	3456-096-K
Collagen I Cell Invasion Assay	3457-096-K
Collagen IV Cell Invasion Assay	3458-096-K

Tumor Spheroid Assays

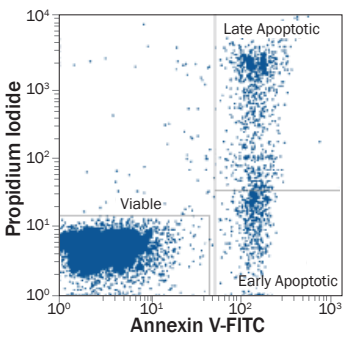
Cultrex® Tumor Spheroid Assays	Catalog #
3D Spheroid Fluorometric Proliferation/Viability Assay	3510-096-K
3D Spheroid Colorimetric Proliferation/Viability Assay	3511-096-K
3D Spheroid Cell Invasion Assay	3500-096-K

Apoptosis and Cell Death Assays

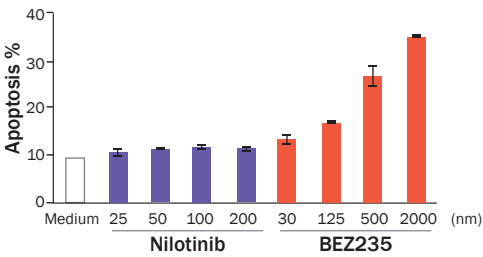
Apoptosis and Cell Death Assays	Catalog #
Annexin V-FITC Apoptosis Detection Kit	4830-01-K
TdT <i>In Situ</i> Apoptosis Detection Kit	4810-30-K
Caspase-3 Colorimetric Assay Kit	BF3100
Proteome Profiler™ Human Apoptosis Array Kit	ARY009



FBS Stimulates Migration of HT1080 Human Fibrosarcoma. The HT1080 human fibrosarcoma cells were either untreated (yellow bars) or treated with 10% fetal bovine serum (FBS; green bars). The histogram quantifies migration of HT1080 cells plated on BME, Laminin I, Collagen I, or Collagen IV. Migration was quantified using the respective Cultrex® Cell Invasion Assay Kits.



Detection of Apoptotic Cells using the TACS Annexin-V-FITC Apoptosis Kit. Thymocytes were treated with 100 nM dexamethasone for 15.5 hours and then stained using Annexin V-FITC and propidium iodide provided in the Annexin V-FITC Apoptosis Detection Kit (Catalog # 4830-01-K). The combination of Annexin V-FITC and propidium iodide allows for the distinction between early apoptotic cells (Annexin V-FITC positive), late apoptotic and/or necrotic cells (Annexin V-FITC and propidium iodide positive), and viable cells (unstained). *Analysis courtesy of Dr. C.M. Knudson, Howard Hughes Medical Institute, St. Louis, MO.*



Toxicity in Ph+ Chronic Myeloid Leukemia Cell Line. The Ph+ chronic myeloid leukemia cell line, SUP-B15, were incubated with different concentrations of the small molecule inhibitors, nilotinib or BEZ235, for 48 hours. Cell apoptosis was assessed using the TACS Annexin-V-FITC Apoptosis Kit (Catalog # 4830-01-K). *Adapted from Ding J., et al. (2013) PLoS ONE 8:e83510.*

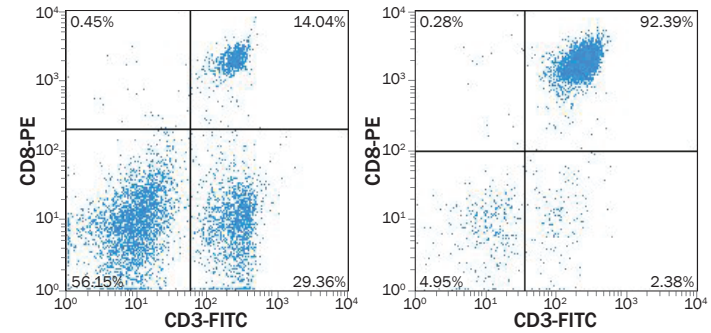
INVESTIGATE

Cancer Stem Cell Immunotherapy

Cell Selection Kits for Adoptive Transfer

Isolate the cells you need for your adoptive cell transfer experiments with our MagCelect™ Cell Selection Kits. Using these kits, unwanted cells are magnetically tagged with a biotinylated antibody cocktail and streptavidin ferrofluid. The cell suspension is placed into a magnetic field, and the desired, untouched cell of choice is harvested by aspiration.

MagCelect™ Kit Name	Species	Catalog #
Regulatory T Cell Isolation Kit	Human	MAGH104
	Mouse	MAGM208
CD4+ T Cell Isolation Kit	Human	MAGH102
	Mouse	MAGM202
CD8+ T Cell Isolation Kit	Human	MAGH112
	Mouse	MAGM203
Memory CD4+ T Cell Isolation Kit	Human	MAGH116
	Mouse	MAGM206
Naïve CD4+ T Cell Isolation Kit	Human	MAGH115
	Mouse	MAGM207
Naïve CD8+ T Cell Isolation Kit	Mouse	MAGM203
NK Cell Isolation Kit	Human	MAGH109
	Mouse	MAGM210



Enrichment of Mouse Splenocytes using the MagCelect™ Mouse CD8+ T Cell Isolation Kit (Catalog # MAGM203)

View all kits | rndsystems.com/cellisolation

Immune Cell Differentiation and Expansion Kits

CellIXvivo™ Immune Cell Differentiation Kits are an efficient, simple, and reliable way to differentiate lymphoid and myeloid immune cells ex vivo. These kits contain optimized combinations of R&D Systems® high-quality cytokines and antibodies.

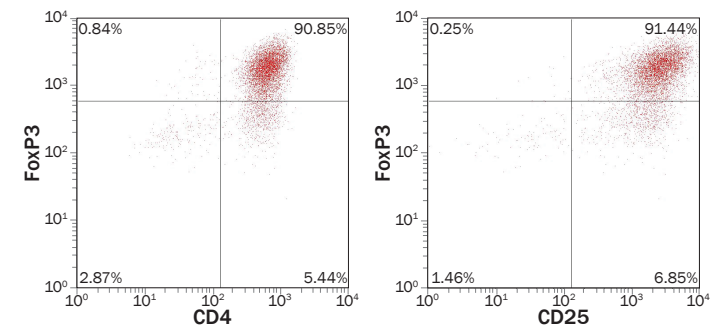
Benefits

- Guaranteed to produce consistent and reliable results
- Cost-effective and time-saving
- Conveniently packages all reagents needed for immune cell differentiation or expansion

CellIXvivo™ Kit	Species	Catalog #
B Cell Expansion Kit	Human	CDK005
Th1 Cell Differentiation Kit	Human	CDK001
Th2 Cell Differentiation Kit	Human	CDK002
Th17 Cell Differentiation Kit	Human	DK003B
Treg Cell Differentiation Kit	Human	CDK006
	Mouse	CDK007
Monocyte-derived Dendritic Cell Differentiation Kit	Human	CDK004
	Mouse	coming soon
M1 Macrophage Differentiation Kit	Human	CDK012
M2 Macrophage Differentiation Kit	Human	CDK013

Features

- Includes optimized combinations of R&D Systems® high-quality proteins and antibodies
- Simple and optimized protocols
- Quality controlled for consistency and reliability
- Does not require specialized instrumentation



Differentiation of CD4+ T Cells into Treg Cells using the CellIXvivo™ Human Treg Cell Differentiation Kit (Catalog # CDK006)

View all kits | rndsystems.com/immunotherapy

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 Transition. 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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