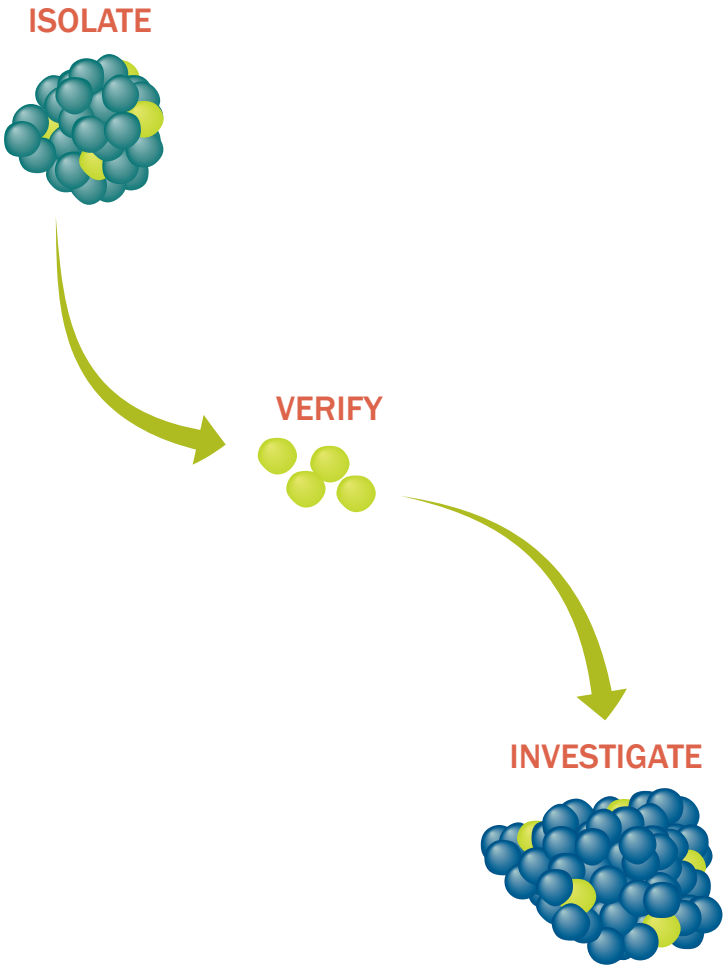
# Cancer Stem Cells

## Isolate, Verify, and Investigate




R&D SYSTEMS®

# The CSC Hypothesis

Like all stem cells, oncogenically transformed cancer stem cells (CSCs) are defined by their ability for self-renewal and multipotency. The CSC hypothesis states that, although CSCs represent a rare population of cells within a tumor, their high tumorigenic capacity drives tumorigenesis. Due to their intrinsic stem cell-like properties, CSC proliferation generates more CSCs and all the differentiated cell types that compose the bulk of the tumor.

Differentiated cancer cells have been shown to proliferate at a faster rate than CSCs but have little tumor-initiating potential. Because CSCs exhibit increased resistance to toxic and chemical insults, this specific subpopulation of cells is believed to underlie resistance to chemotherapy and disease relapse. In fact, the CSC model suggests that all CSCs must be eradicated to eliminate a tumor and prevent its recurrence.

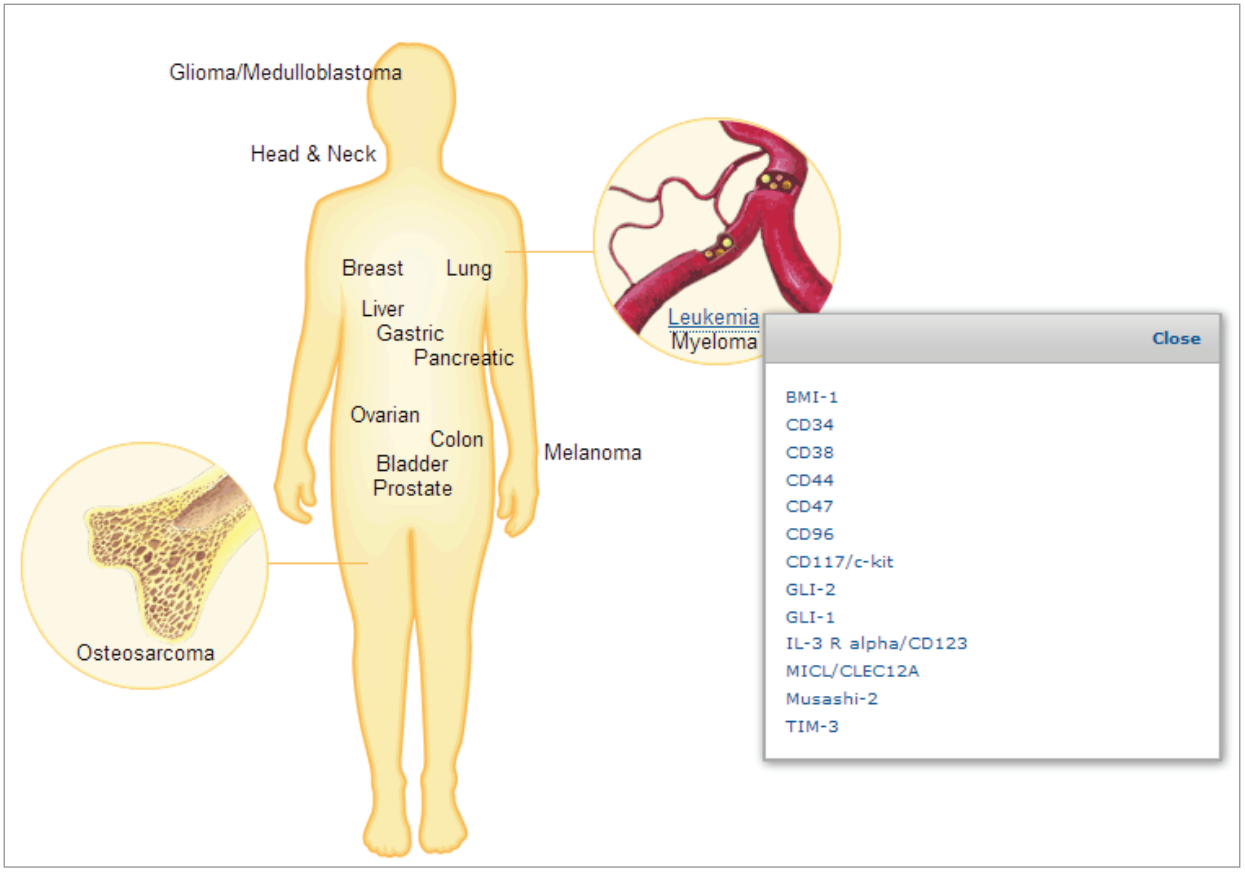
Unlike the differentiated cancer cells in a tumor, xeno-transplantation of human CSCs gives rise to new tumors in immunodeficient animal models. Although inconsistencies in these studies have questioned the validity of the CSC hypothesis, it is likely that variable findings result from differences in assay sensitivity and quantification methodology. An overriding experimental limitation for CSC researchers is the ability to isolate CSCs and verify CSC marker expression. To address this need, we present our product offering for the isolation, verification, and investigation of CSCs.

learn more | RnDSystems.com/CSCProducts

### Malignancy-specific Markers Available for:

- Bladder Cancer
- Breast Cancer
- Colon Cancer
- Gastric Cancer
- Glioma/Medulloblastoma
- Head & Neck Cancer
- Leukemia
- Liver Cancer
- Lung Cancer
- Melanoma
- Myeloma
- Osteosarcoma
- Ovarian Cancer
- Pancreatic Cancer
- Prostate Cancer

Check out the interactive CSC marker page on our website | RnDSystems.com/CSCi



# ISOLATE

# MagCellect™ Cell Selection Kits for Cancer Stem Cell Research

## MagCellect Human CD24$^{-}$CD44$^{+}$ Breast Cancer Stem Cell Isolation Kit

The MagCellect Human CD24$^{-}$CD44$^{+}$ Breast Cancer Stem Cell Isolation Kit (Catalog # MAGH111) was designed to isolate a rare population of human breast cancer cells that have the unique ability to form new tumors in mice.[1] This kit utilizes a two-step procedure that combines negative and positive selection techniques. CD24$^{low/-}$ cells are initially enriched by negative selection whereby unwanted CD24$^{+}$ cells are tagged and magnetically removed. CD44$^{+}$ cells are subsequently isolated from the CD24$^{low/-}$ population by positive selection using a biotinylated human CD44 antibody, streptavidin-conjugated magnetic beads, and a MagCellect Magnet (Catalog # MAG997, or equivalent). The efficiency of enrichment can be assessed by staining the recovered cells with fluorochrome-conjugated anti-human CD24 and CD44 detection antibodies provided in the kit.

Reference 1. Al-Hajj, M. *et al.* (2003) Proc. Natl. Acad. Sci. USA **100**:3983.

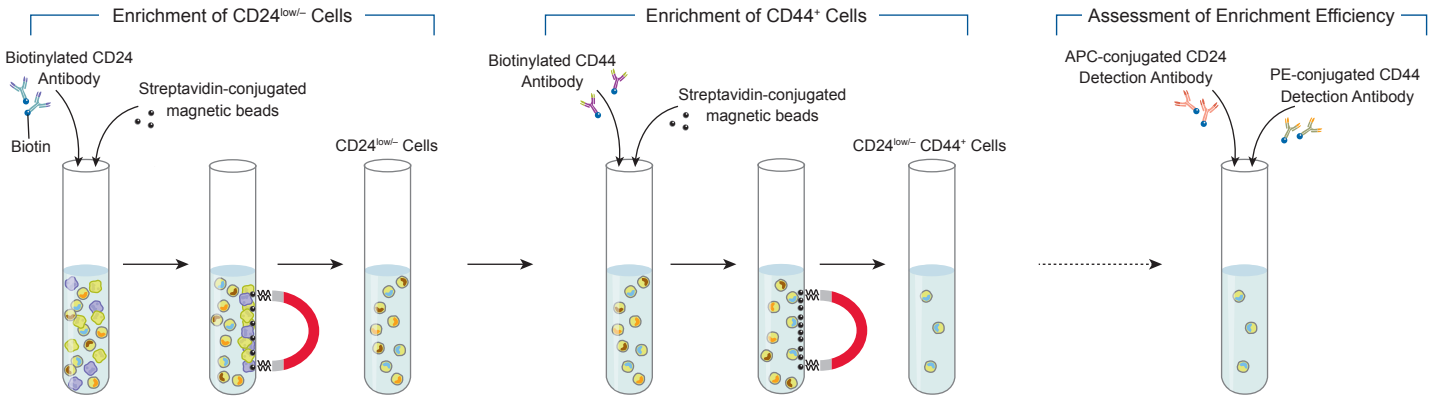
### Features

- No cell damage induced by beads or ferrofluids
- 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

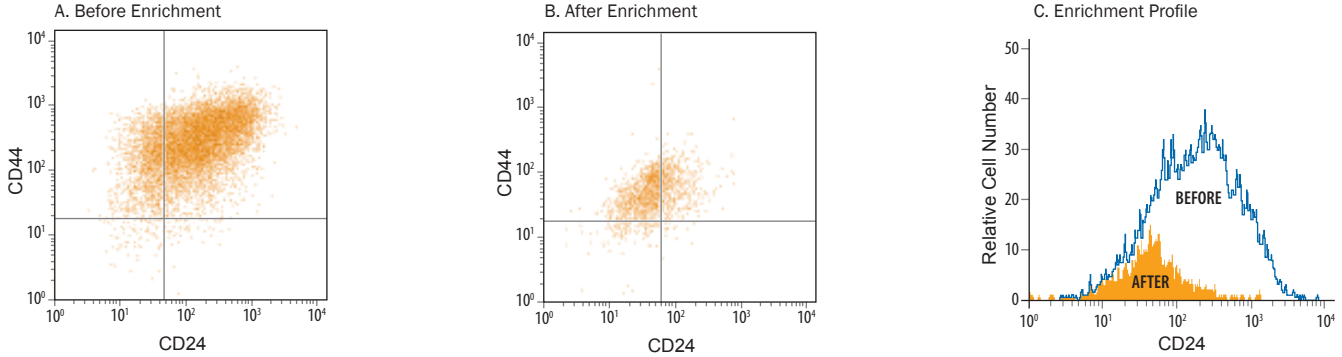
### MagCellect Kit Catalog # MAGH111

| Kit | Contents |
|---|---|
| MagCellect CD24$^{-}$CD44$^{+}$ Breast Cancer Stem Cell Isolation Kit | Biotinylated CD24 Selection Antibody, Biotinylated CD44 Selection Antibody, APC-conjugated CD24 Detection Antibody, PE-conjugated CD44 Detection Antibody, Streptavidin Ferrofluid, 10X MagCellect Buffer |

### Assay Principle




**Analysis of CD24$^{low/-}$CD44$^{+}$ Cells using the MagCellect Human CD24$^{-}$CD44$^{+}$ Breast Cancer Stem Cell Isolation Kit.** Undesired CD24$^{+}$ cells are bound by a biotinylated anti-human CD24 antibody and captured by streptavidin-conjugated magnetic particles. These cells are then isolated using a MagCellect Magnet (Catalog # MAG997, or equivalent), and an enriched CD24$^{low/-}$ population is aspirated from the sample solution. CD44$^{+}$ cells are subsequently labeled with a biotinylated anti-human CD44 antibody and magnetically tagged with streptavidin-conjugated magnetic particles. CD24$^{low/-}$CD44$^{+}$ cells are captured by applying the MagCellect Magnet, or any compatible system, and undesired cells are aspirated from solution.




**Analysis of CD24$^{low/-}$CD44$^{+}$ Cells Isolated using the MagCellect Human CD24$^{-}$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 MagCellect CD24$^{-}$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).

# VERIFY

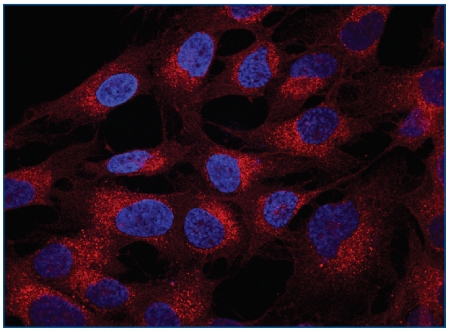
## Products for Cancer Stem Cell Markers

| Molecule | Recombinant & Natural Proteins | Antibodies | ELISAs, Cell Selection & Detection Kits & Reagents |
|---|---|---|---|
| **Bladder Cancer Stem Cell Markers** | | | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| CD44 | H M R P | H M R Ca | H **H** |
| CD47 | H M | H M | |
| CEACAM-6/CD66c | H | H | H |
| **Breast Cancer Stem Cell Markers** | | | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| BMI-1 | | H | |
| CD24 | H | H | **H** |
| CD44 | H M R P | H M R Ca | H **H** |
| Connexin 43/GJA1 | | H M R B Ca Ch Pr Z Fi GP S X | |
| CXCR1/IL-8 RA | | H | |
| CXCR4 | | H M F | **H** |
| DLL4 | H M | H M | M |
| EpCAM/TROP1 | H | H | H **H** |
| ErbB2/Her2 | H | H M | H **H** |
| GLI-1 | | H M | |
| GLI-2 | | H M | |
| IL-1α/IL-1F1 | H M R CR P | H M R CR P | H M R |
| IL-6 Rα | H M | H M | H M |
| Integrin α6/CD49f | | H M B | |
| PON1 | | H M | H |
| PTEN | H | H M R | |
| **Colon Cancer Stem Cell Markers** | | | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| ALCAM | H M | H M R | H M |
| CD44 | H M R P | H M R Ca | H **H** |
| DPPIV/CD26 | H M | H M | H M |
| EpCAM/TROP1 | H | H | H **H** |
| GLI-1 | | H M | |
| Musashi-1 | | H | |
| **Gastric Cancer Stem Cell Markers** | | | |
| CD44 | H M R P | H M R Ca | H **H** |
| DLL4 | H M | H M | M |
| **Glioma/Medulloblastoma Cancer Stem Cell Markers** | | | |
| A20/TNFAIP3 | H | H | |
| ABCG2 | | H | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| BMI-1 | | H | |
| CD15/Lewis X | | H | |
| CD44 | H M R P | H M R Ca | H **H** |
| CX3CL1/Fractalkine | H M R | H M R | H M R |
| CX3CR1 | | H M | |
| CXCR4 | | H M F | **H** |

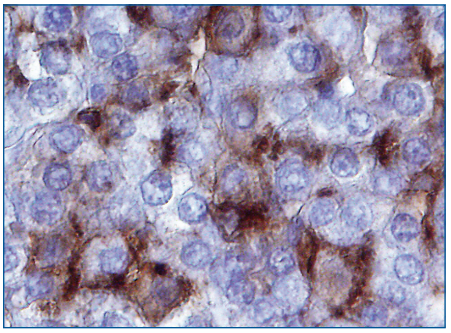
| Molecule | Recombinant & Natural Proteins | Antibodies | ELISAs, Cell Selection & Detection Kits & Reagents |
|---|---|---|---|
| HIF-2α/EPAS1 | | H M R | H R |
| IL-6 Rα | H M | H M | H M |
| Integrin α6/CD49f | | H M B | |
| L1CAM | H M | H M | |
| Musashi-1 | | H | |
| c-Myc | | H | |
| Nestin | | H M R | |
| Podoplanin | H M | H M | |
| SOX2 | | H M | |
| **Head & Neck Cancer Stem Cell Markers** | | | |
| ABCG2 | | H | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| BMI-1 | | H | |
| CD44 | H M R P | H M R Ca | H **H** |
| HGF R/c-MET | H M Ca | H M Ca | H M Ca **H** |
| Lgr5/GPR49 | H | | |
| **Leukemia Cancer Stem Cell Markers** | | | |
| BMI-1 | | H | |
| CD34 | | H M R Ca P | **H** |
| CD38 | H M | H M | |
| CD44 | H M R P | H M R Ca | H **H** |
| CD47 | H M | H M | |
| CD96 | M | H M | |
| CD117/c-kit | H M | H M | H |
| GLI-1 | | H M | |
| GLI-2 | | H M | |
| IL-3 Rα/CD123 | H M | H M | |
| MICL/CLEC12A | | H M | |
| Musashi-2 | | H | |
| TIM-3 | H M CM | H M | H |
| **Liver Cancer Stem Cell Markers** | | | |
| α-Fetoprotein | | H M | H |
| Aminopeptidase N/ANPEP | H M | H M | H |
| CD45 | H M | H M | **H** |
| CD90/Thy1 | M | H M | |
| NF2/Merlin | | H | |
| **Lung Cancer Stem Cell Markers** | | | |
| ABCG2 | | H | |
| Aldehyde Dehydrogenase 1-A1/ALDH1A1 | H | H | |
| CD90/Thy1 | M | H M | |
| CD117/c-kit | H M | H M | H |
| EpCAM/TROP1 | H | H | H **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

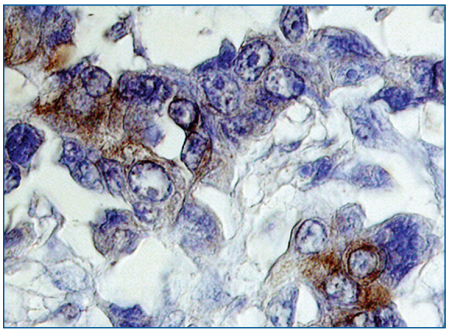
| Molecule | Recombinant & Natural Proteins | Antibodies | ELISAs, Cell Selection & Detection Kits & Reagents |
|---|---|---|---|
| **Melanoma Cancer Stem Cell Markers** | | | |
| ABCB5 | | H | |
| ABCG2 | | H | |
| ALCAM | H M | H M R | H M |
| MS4A1/CD20 | | H | |
| Nestin | | H M R | |
| NGF R/TNFRSF16 | H M | H M | H M |
| **Myeloma Cancer Stem Cell Markers** | | | |
| ABCB5 | | H | |
| CD19 | | H M R | |
| CD27/TNFRSF7 | H M R | H M | M |
| CD38 | H M | H M | |
| MS4A1/CD20 | | H | |
| Syndecan-1 | H M | H M | H |
| **Osteosarcoma Cancer Stem Cell Markers** | | | |
| α-Methylacyl-CoA Racemase/ AMACR | | H | |
| ABCG2 | | H | |
| CD44 | H M R P | H M R Ca | H **H** |
| Endoglin/CD105 | H M R P | H M | H M **H** |
| Nestin | | H M R | |
| STRO-1 | | H | |
| **Ovarian Cancer Stem Cell Markers** | | | |
| CD44 | H M R P | H M R Ca | H **H** |
| CD117/c-kit | H M | H M | H |
| Endoglin/CD105 | H M R P | H M | H M **H** |
| **Pancreatic Cancer Stem Cell Markers** | | | |
| Aldehyde Dehydrogenase 1-A1/ ALDH1A1 | H | H | |
| BMI-1 | | H | |
| CD24 | H | H | **H** |
| CD44 | H M R P | H M R Ca | H **H** |
| CXCR4 | | H M F | **H** |
| EpCAM/TROP1 | H | H | H **H** |
| PON1 | | H M | H |
| **Prostate Cancer Stem Cell Markers** | | | |
| α-Methylacyl-CoA Racemase/ AMACR | | H | |
| ABCG2 | | H | |
| ALCAM | H M | H M R | H M |
| Aldehyde Dehydrogenase 1-A1/ ALDH1A1 | H | H | |
| BMI-1 | | H | |
| CD44 | H M R P | H M R Ca | H **H** |
| CD151 | | H | |
| c-Myc | | H | H |
| TRA-1-60(R) | | H | |



**STRO-1 in the Human MG-63 Cell Line.** Stromal Cell Precursor Surface Antigen (STRO-1) was detected in immersion-fixed MG-63 human osteosarcoma cells using a Mouse Anti-Human STRO-1 Monoclonal Antibody (Catalog # MAB1038). The cells were stained using the NorthernLights™ 557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL007; red), and the nuclei were counterstained with DAPI (blue). Specific staining was localized to the cell surface and the cytoplasm.



**Aminopeptidase N/ANPEP in Human Liver Cancer Tissue.** Aminopeptidase N/ANPEP was detected in immersion-fixed paraffin-embedded sections of human liver cancer tissue using a Sheep Anti-Human Aminopeptidase N/ANPEP Antigen-affinity Purified Polyclonal Antibody (Catalog # AF3815). The tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS019; brown) and counterstained with hematoxylin (blue). Specific labeling was localized to bile canaliculi.



**ErbB2/Her2 in Human Breast Cancer Tissue.** ErbB2/ Her2 was detected in immersion-fixed paraffin-embedded sections of human breast cancer tissue using a Mouse Anti-Human ErbB2 Monoclonal Antibody (Catalog # MAB11291). The tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS002; brown) and counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasm of cancer cells.

# INVESTIGATE

## Tocris Products for Cancer Stem Cell 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] 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 Tocris.com to explore the variety of other molecules and screening compound libraries.

| 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 | | |
| *endo*-IWR 1 | Increases Axin2 protein levels | 3532 |
| *exo*-IWR 1 | Negative control for *endo*-IWR 1 | 3947 |
| ICG 001 | Inhibits TCF/β-catenin-mediated transcription | 4505 |
| IWP 2 | Inactivates Porcupine (Porcn); selectively inhibits palmitoylation of Wnt | 3533 |
| IWP L6 | Porcn inhibitor | 4992 |
| PNU 74654 | Inhibits the interaction between β-catenin and Tcf4 | 3534 |
| WIKI4 | Tankyrase inhibitor | 4855 |
| Hedgehog Pathway Inhibitors | | |
| Ciliobrevin A | Inhibits ciliogenesis | 4529 |
| Cyclopamine | Smoothened 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 | | |
| BIBR 1532 | Telomerase Inhibitor | 2981 |
| Costunolide | Telomerase Inhibitor | 2483 |
| TMPyP4 tosylate | Telomerase Inhibitor | 4253 |
| Cyclophosphamide | Inhibits ALDH1 through its degradation product acrolein | 4091 |

References

1. Takebe, N. *et al.* (2011) Nat. Rev. Clin. Oncol. **8**:97.

2. Wang, K. *et al.* (2013) Int. J. Nanomedicine **8**:899.

# References for R&D Systems Cancer Stem Cell-related Products


1. Baccelli, I. *et al.* (2013) Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat. Biotechnol. **31**:539.

   Sheep Anti-Human CD47 Antigen-affinity Purified Polyclonal Antibody (Catalog # AF4670)

   Mouse Anti-Human CD44v6 Monoclonal Antibody (Catalog # BBA13)

   **Sample**: Human breast cancer circulating tumor cell (CTC)-induced xenograft
   **Application**: Immunohistochemistry

   APC-conjugated Mouse Anti-Human HGF R/c-MET Monoclonal Antibody (Catalog # FAB3582A)

   **Sample**: Human breast cancer CTCs
   **Application**: Flow cytometry

2. Lotti, F. et al. (2013) Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. J. Exp. Med. **210**:2851.

   Fluorescein-conjugated Mouse Anti-Human EpCAM/TROP1 Monoclonal Antibody (Catalog # FAB9601F)

   APC-conjugated Mouse Anti-Human IL-17 R Monoclonal Antibody (Catalog # FAB177A)

   PE-conjugated Mouse Anti-Human PDGF Rα Monoclonal Antibody (Catalog # FAB1264P)

   **Sample**: Human colorectal cancer patient tumor cells
   **Application**: Flow cytometry

3. Hirsch, H.A. *et al.* (2013) Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. Proc. Natl. Acad. Sci. USA **110**:972.

   Human CD44$^+$ Cancer Stem Cells PlusCellect™ Kit (Catalog # PLS4948)

   **Sample**: Human breast cancer tissues; MCF10A-ER-Src human breast epithelial cells
   **Application**: Cancer stem cell isolation

   Human/Mouse Phospho-STAT3 (Y705) Cell-Based ELISA (Catalog # KCB4607)

   **Sample**: Human breast cancer tissues
   **Application**: Phospho-STAT3 measurement

4. Bareiss, P.M. *et al.* (2013) *SOX2* expression associates with stem cell state in human ovarian carcinoma. Cancer Res. **73**:5544.

   Goat Anti-Human SOX2 Antigen-affinity Purified Polyclonal Antibody (Catalog # AF2018)

   **Sample**: Caov3 human ovarian adenocarcinoma cell line-derived tumors
   **Application**: Immunohistochemistry

5. Gassenmaier, M. *et al.* (2013) CXC chemokine receptor 4 is essential for maintenance of renal cell carcinoma-initiating cells and predicts metastasis. Stem Cells **31**:1467.

   Mouse Anti-Human CXCR4 (Fusin) Monoclonal Antibody (Catalog # MAB172-100)

   **Sample**: Human primary renal cell carcinoma tumor cells
   **Application**: Immunohistochemistry

6. Volonté, A. *et al*. (2013) Cancer-initiating cells from colorectal cancer patients escape from T cell-mediated immunosurveillance *in vitro* through membrane-bound IL-4. J. Immunol. **192**:523.

   Mouse Anti-Human/Mouse SOX2 Monoclonal Antibody (Catalog # MAB2018)

   Mouse Anti-Human CD24 Monoclonal Antibody (Catalog # MAB5248)

   **Sample**: Colorectal cancer (CRC)-derived spheroid cell cultures
   **Application**: Immunofluorescence and cytofluorimetric analysis

   Mouse Anti-Human IL-4 Rα Monoclonal Antibody (Catalog # MAB230)

   **Sample**: Cancer-initiating cells from CRC patients
   **Application**: Blocking/Neutralization

7. Hage, C. *et al.* (2013) The novel c-Met inhibitor cabozantinib overcomes gemcitabine resistance and stem cell signaling in pancreatic cancer. Cell Death Dis. **9**:e627.

   Proteome Profiler™ Human Pluripotent Stem Cell Array Kit (Catalog # ARY010)

   Proteome Profiler Human Apoptosis Array Kit (Catalog # ARY009)

   **Sample**: BxPc-3 human pancreatic adenocarcinoma cell line
   **Application**: Profiling of protein expression

   Rabbit Anti-Human/Mouse Cleaved Caspase-3 (Asp175) Monoclonal Antibody (Catalog # MAB835)

   **Sample**: Pancreatic ductal adenocarcinoma patient-derived primary spheroidal cultures
   **Application**: Immunohistochemistry

   Rabbit Anti-Human Survivin Antigen-affinity Purified Polyclonal Antibody (Catalog # AF886)

   **Sample**: BxPc-3 human pancreatic adenocarcinoma cell line
   **Application**: Western blot

8. Lepiller, Q. *et al.* (2013) HCMV activates the IL-6-JAK-STAT3 axis in HepG2 cells and primary human hepatocytes. PLoS One **8**:e59591.

   StemXVivo™ Serum-Free Tumorsphere Media (Catalog # CCM012)

   **Sample**: HepG2 human hepatocellular carcinoma cell line
   **Application**: Tumorsphere assay

   Human IL-6 Quantikine® ELISA Kit (Catalog # D6050)

   **Sample**: HepG2 human hepatocellular carcinoma cell line and primary human hepatocytes
   **Application**: Cell culture

On the Cover

**A. Tumorsphere Formation using the StemXVivo™ Serum-Free Tumorsphere Media.** The MCF-7 human breast cancer cell line was cultured for 7 days in StemXVivo Serum-Free Tumorsphere Media (Catalog # CCM012) to induce tumorsphere formation. The morphology of MCF-7 tumorspheres was documented using a CloneSelect™ Imager from Molecular Devices LLC.

**B. EpCAM/TROP1 in the HT-29 Human Cell Line.** Epithelial Cellular Adhesion Molecule (EpCAM/TROP1) was detected in immersion-fixed HT-29 human colon adenocarcinoma cells using a Mouse Anti-Human EpCAM/TROP1 Monoclonal Antibody (Catalog # MAB960). The cells were stained using the NorthernLights 493-conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL009; green) and the nuclei were counterstained with DAPI (blue). Specific staining was localized to the cell surface and the cytoplasm.

**C. Intracellular Detection of BMI-1 by Flow Cytometry.** The HeLa human cervical epithelial carcinoma cell line was stained with an APC-conjugated Mouse Anti-Human BMI-1 Monoclonal Antibody (Catalog # IC33341A; filled histogram) or an APC-conjugated Mouse $IgG_{2A}$ Isotype Control Antibody (Catalog # IC003A; open histogram).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MA_2.14_CSC Markers_AARC_951


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