

Cancer Research

Products for Cell Isolation, Culture and Analysis

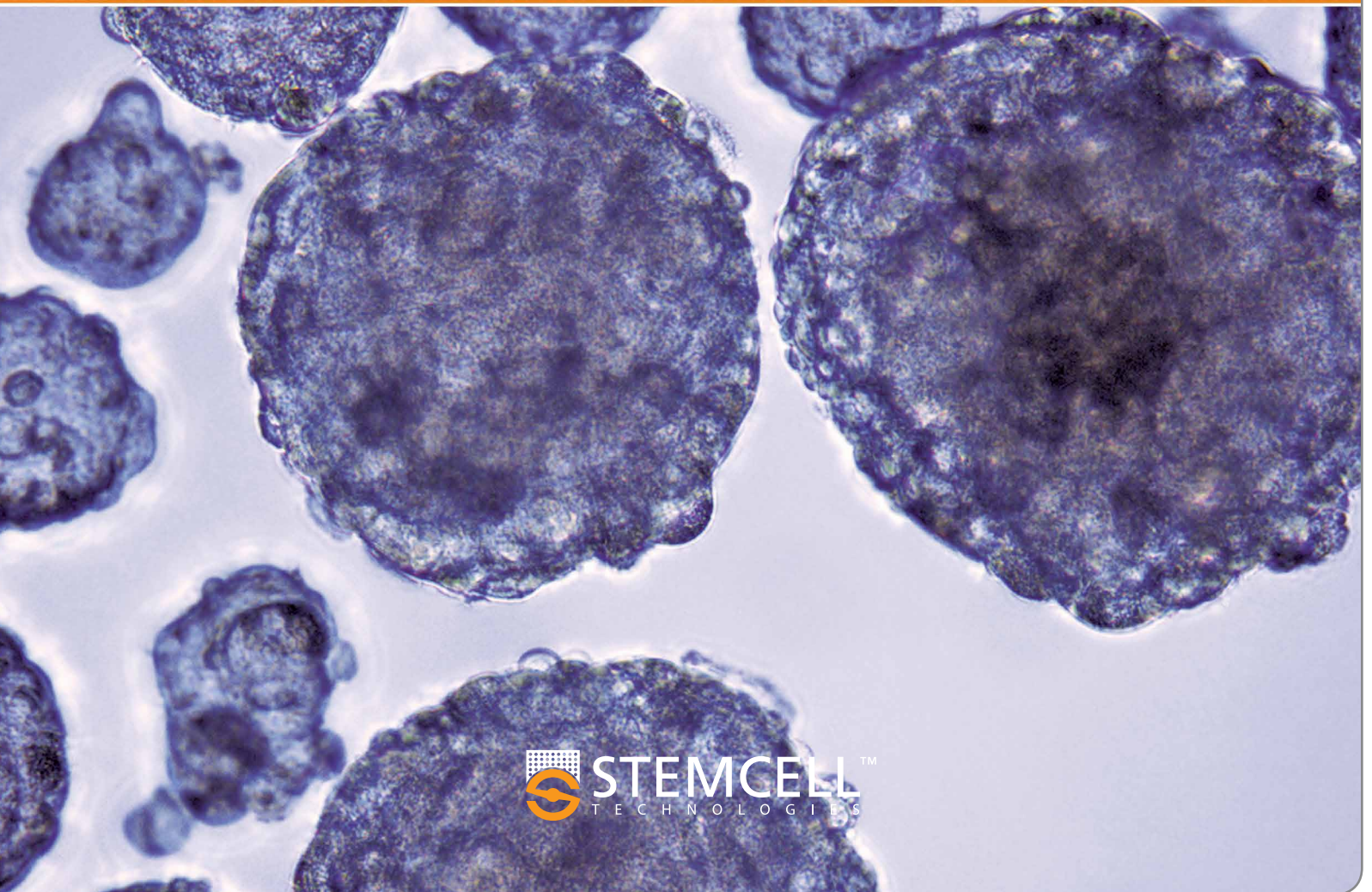
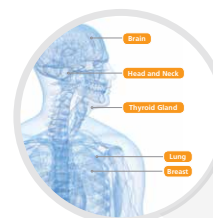
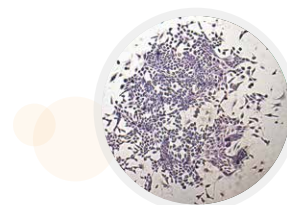
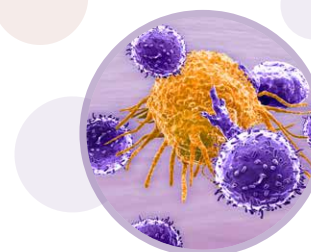
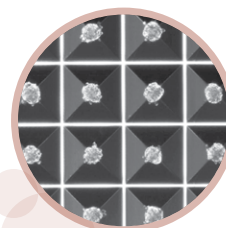
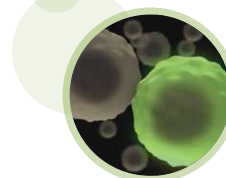


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Front cover: MCF7 tumorspheres cultured for 8 days in MammoCult™. Learn more on page 5.

Cancer Research with 3D Spheroids

3D Cell Culture

Three dimensional (3D) cell culture is more physiologically relevant than traditional adherent or single cell culture methods. It provides a better representation of the in vivo microenvironment and is widely thought to be more predictive of disease state and drug response.

3D culture systems can be used for many applications, including disease modeling,^{1,2} drug screening,³ cell signaling and differentiation studies,⁴ and 3D tissue engineering.⁵

3D Spheroids with AggreWell™

Easily generate large numbers of uniform 3D cancer spheroids with AggreWell™ plates. Each well contains a standardized array of microwells, allowing the production of highly uniform spheroids in just 24-48 hours. Spheroid size can be easily controlled by adjusting the cell seeding concentration.

AggreWell™ plates are compatible with a variety of cell types, including breast cancer,⁶ prostate cancer,⁷ colon cancer,⁷ liver cancer^{3,8,9} glioblastoma cell lines¹⁰ and more.

AggreWell™ plates are available in 2 sizes of microwells and multiple plate formats to fit your research needs.

AggreWell™ Products for Spheroid Production

PRODUCT	SIZE OF MICROWELL	PLATE FORMAT	CATALOG #
AggreWell™400	400 µm	24-well plate	27845/27945
		6-well plate	34421/34425
AggreWell™800	800 µm	24-well plate	34811/34815
		6-well plate	34821/34825
AggreWell™ Rinsing Solution	Required for use with AggreWell™ plates to ensure optimal performance.		07010



AggreWell™ Plate with Microwells.

Why Use AggreWell™?

- High yield of spheroids
- Uniform spheroids with consistent size & shape
- Low cost-per-spheroid
- Easy to use
- Reproducible
- Compatible with a variety of cell types

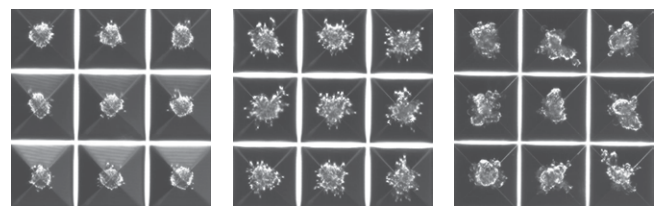


Figure 1. Cancer cell lines form uniform spheroids in AggreWell™. Shown are DU145 (prostate), A549 (lung) and MCF7 (breast) cancer cell lines, 500 cells per microwell in AggreWell™400.

For more information about AggreWell™, please visit us at www.stemcell.com or email us at aggrewell@stemcell.com

Cancer Stem Cell Research

ALDEFLUOR™ and ALDH^{br} Cells

The ALDEFLUOR™ fluorescent reagent system (Catalog #01700) has supported more than 2000 publications by detecting Aldehyde Dehydrogenase-bright (ALDH^{br}) cells in over 80 distinct cell types.

ALDEFLUOR™ was originally designed to identify a unique population of human stem and progenitor hematopoietic cells that exhibit elevated levels of ALDH expression. ALDEFLUOR™ is a non-immunological reagent system that enables identification, quantification and isolation of viable cells based on intracellular ALDH activity levels, rather than on cell surface phenotype. The utility of ALDH activity as a marker to identify multipotential hematopoietic stem and progenitor cells has subsequently been extended to other applications, where it has been recognised as a useful marker for putative stem and progenitor cells in a variety of healthy and cancerous tissues.

A selected list of publications using ALDEFLUOR™ for cancer research is available at www.stemcell.com/ALDReferences.

ALDEFLUOR™ in Cancer Research

While not a universal marker for cancer stem cells in any tissue, ALDH activity has proven a useful marker for both normal and malignant cells with stem-like properties in a great variety of tissues.¹¹ Increased ALDH expression has been found in multiple myeloma and acute myeloid leukemia (AML),¹²⁻¹⁴ prostate,¹⁵ colon,^{16,17} head and neck,^{18,19} thyroid gland,²⁰ breast,²¹⁻²³ liver,²⁴ ovarian,²⁵ cervical,²⁶ bladder,²⁷ brain²⁸ and lung²⁹ cancers. Studies have also shown a correlation between the ALDH phenotype and poor prognosis in various cancers.^{25, 30,31}



REFERENCES

Selected Cancer References
www.stemcell.com/ALDReferences



VIDEO

The Basic FACS About ALDEFLUOR™
www.stemcell.com/BasicFACS

Why Use ALDEFLUOR™?

- No antibodies required
- Can be used with multiple species and cell types
- Compatible with immunophenotyping
- Compatible with standard cell sorters or analyzers
- Has supported 2000+ publications
- Highly reproducible results

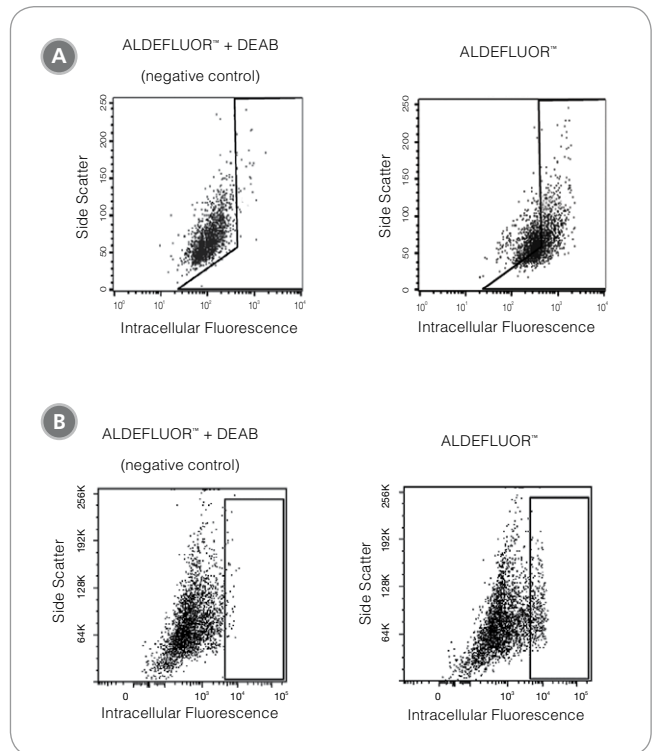


Figure 2. ALDEFLUOR™ is compatible with a variety of cell types, including cancer cell lines and primary tissue samples. (A) K562 breast cancer cells stained with ALDEFLUOR™. (B) Primary normal human mammary epithelial samples stained with ALDEFLUOR™.

ALDEFLUOR™ is a trademark of Aldagen Inc.

Breast Cancer Research

Understanding the organization of the mammary epithelial cell hierarchy is important for identifying the cell-of-origin for different types of human breast tumours, characterizing the cells that drive tumor growth, and understanding how different oncogenic mutations influence homeostasis within the normal mammary epithelium. Adherent and non-adherent in vitro colony-forming assays are valuable approaches for interrogating the functional heterogeneity present within normal human and mouse mammary tissue, within mammary tissue of genetically modified mice, and human breast tumor samples.

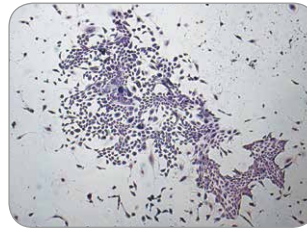
Culture and assay normal and malignant mammary cells using the defined MammoCult™ and EpiCult™ cell culture media. EpiCult™ media support the growth of human- and mouse-derived mammary epithelial cells in adherent culture. MammoCult™ is the most-published commercially-available medium for culture of human mammospheres derived from normal human mammary tissue and tumorsphere formation from multiple breast cancer cell lines.

Products for the Assay and Culture of Primary Mammary Progenitor Cells

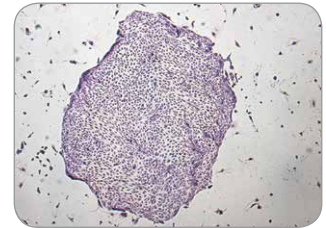
PRODUCT	APPLICATION	CATALOG #
EpiCult™-B (Human)	Colony-forming cell assay for differential assessment of progenitor content	05601
EpiCult™-B (Mouse)	Colony-forming cell assay for assessment of progenitor content	05610
EpiCult™-C (Human)	Short-term culture of primary human mammary epithelial cells	05630
MammoCult™	Generation of robust human mammospheres and tumorspheres in optimized and defined culture conditions	05620
ALDEFLUOR™	Identification, enumeration and isolation of viable normal and cancer cells on the basis of their ALDH activity	01700

Mammary Epithelial Colony and Sphere Images

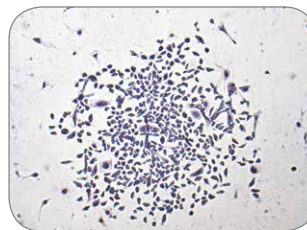
Examples of Colonies Derived From Human and Mouse Mammary Epithelial Progenitors



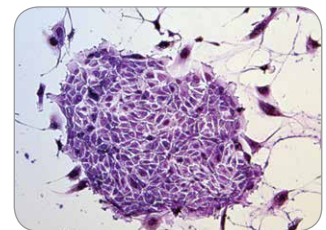
Normal Human Mixed-Lineage Colony



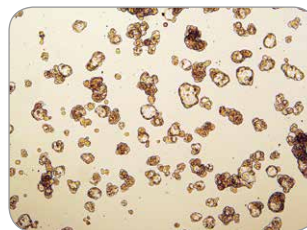
Normal Human Pure Luminal Cell Colony



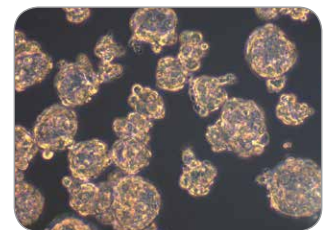
Normal Human Pure Myoepithelial Cell Colony



Normal Mouse Mammary Epithelial Colony



Mammospheres from Primary Normal Human Mammary Epithelial Cells



Tumorspheres from MCF7 Human Breast Cancer Cell Line

MammoCult™ can be used to culture tumorspheres from primary breast cancer tissue and a variety of breast cancer cell lines including MCF7, MCF10A, SKBR3, MDA-MB-231, AU565, SUM149 and BT474. Learn more at www.stemcell.com/MammoCult.



WALLCHART

SnapShot: Breast Cancer

www.stemcell.com/BreastCancerWallchart

Brain Tumor Research

Multipotent neural stem-like cells, known as brain tumor stem cells (BTSCs) or cancer stem cells, have been identified and isolated from different grades (low and high) and types of brain cancers, including gliomas and medulloblastomas.^{32,33} Similar to neural stem cells (NSCs), these BTSCs exhibit self-renewal, high proliferative capacity and multi-lineage differentiation potential in vitro.

BTSCs can either be cultured as free-floating aggregates (neurospheres) or as an adherent monolayer of cells. For both methods, cells are plated in a defined, serum-free medium in the presence of a mitogenic factor, such as EGF and/or bFGF. In the neurosphere system, cells are cultured in the absence of a culture substrate, which causes the cells to grow as non-adherent clusters - the neurospheres. Importantly, the neurosphere assay may be a clinically relevant functional read-out for the study of BTSCs, with recent research suggesting that renewable neurosphere formation in cultured human glioma samples may be a significant predictor of increased risk of rapid tumor progression and patient death.³⁴⁻³⁶ Adherent monolayer culture has recently been shown to enable pure populations of glioma-derived BTSCs to be expanded in vitro.³⁷

The standardized NeuroCult™ culture system provides a wide range of species-specific media and supplements, for the proliferation and differentiation of human, mouse and rat neural stem and progenitor cells from normal or tumor CNS tissue. Components for all NeuroCult™ media and supplements adhere to STEMCELL Technologies' renowned quality control standards, which include prescreening raw materials before manufacturing, and performance testing in relevant assays.

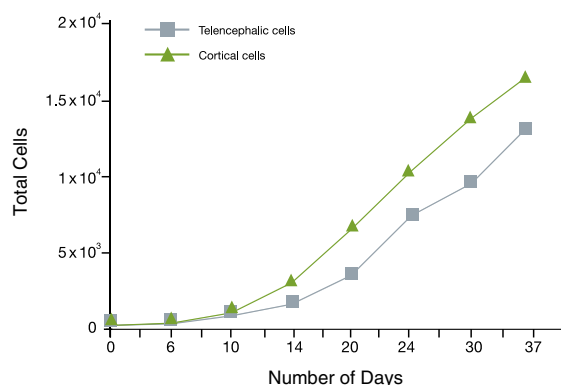


Figure 3. Total cell expansion for fetal human telencephalic and cortical cells cultured as neurospheres with complete NeuroCult™ NS-A Proliferation Medium containing rh EGF, rh bFGF and heparin (n = 2)

NeuroCult™ media and dissociation reagents have been used to:

- Dissociate human glioblastoma and oligodendroglioma samples³⁸
- Culture human glioblastoma-derived³⁹⁻⁴¹ and oligodendroglioma-derived⁴² tumorspheres
- Culture cells obtained from mouse models of medulloblastoma⁴³ and glioma⁴⁴ as tumorspheres
- Differentiate brain tumor stem cells into neurons, astrocytes and oligodendrocytes^{43,44}
- Passage/dissociate tumorspheres⁴⁵

Products for Brain Tumor Stem Cell Research

PRODUCT	CATALOG #
NeuroCult™ NS-A Proliferation Kit (Human)*	05751
NeuroCult™-XF Proliferation Medium*	05761
NeuroCult™ NS-A Differentiation Kit (Human)	05752
NeuroCult™ Proliferation Kit (Mouse)*	05702
NeuroCult™ Differentiation Kit (Mouse)	05704
NeuroCult™ NS-A Proliferation Kit (Rat)*	05771
NeuroCult™ NS-A Differentiation Kit (Rat)	05772

*Requires supplementation with rh EGF (Catalog #78006). When culturing cells obtained from adult mouse, rh bFGF (Catalog #78003) and Heparin (Catalog #07980) are also required.



WALLCHART

SnapShot: Glioblastoma Multifforme

www.stemcell.com/GlioblastomaWallchart

Intestinal Cancer Research

Intestinal epithelial organoid cultures grown using IntestiCult™ Organoid Growth Medium (Mouse) (Catalog #06005) provide a convenient, organotypic *in vitro* model system for investigating intestinal and colonic cancers. Intestinal epithelial organoids incorporate key features that mimic the *in vivo* intestinal physiology, including a functional central lumen surrounded by a polarized epithelial cell layer.⁴⁶ Intestinal organoids contain intestinal stem cells, which both self-renew within this culture system and differentiate to form the paneth cells, goblet cells, enteroendocrine cells and enterocytes that comprise the organoid structure. Organoids can be subjected to a wide variety of treatments including genetic manipulation,⁴⁷ application of inflammatory cytokines or signalling molecules,⁴⁷ co-culture with intraluminal bacteria⁴⁸ and viral infection.⁴⁹ The cellular dynamics and experimental flexibility inherent in this system make organoids a valuable research tool for studying the characteristics of intestinal cancers.

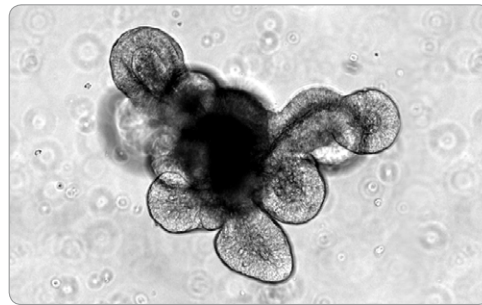


Figure 4. Light microscope visualization of a mouse intestinal epithelial organoid after five days of culture in IntestiCult™ Organoid Growth Medium.



WALLCHART

SnapShot: The Intestinal Crypt
www.stemcell.com/Crypt_Wallchart

Pancreatic Cancer Research

Pancreatic cancer researchers have seen the options for *in vitro* pancreatic cell culture expand over the last several years with the development of protocols for directed differentiation of pluripotent stem cells to multipotent pancreatic progenitor cells and downstream differentiated cell types.⁵⁰⁻⁵² The STEMdiff™ Pancreatic Progenitor Kit (Catalog #05120) is a serum-free, defined medium system that supports efficient and reproducible generation of pancreatic progenitor cells from both human embryonic stem (ES) and induced pluripotent stem (iPS) cells. This kit directs differentiation of ES and iPS cells through definitive endoderm, primitive gut tube and posterior foregut endoderm to pancreatic progenitor cells characterized by expression of key transcription factors, including PDX-1, NKX6.1 and SOX9. The resulting pancreatic progenitor cells are multipotent and can be matured *in vitro* or *in vivo* to endocrine and exocrine pancreatic cells that can be used to investigate characteristics of and potential treatments for pancreatic cancer.

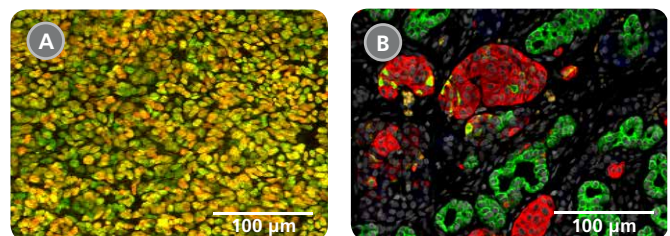


Figure 5. PDX-1⁺/NKX6.1⁺ Pancreatic progenitors mature to form endocrine and exocrine pancreatic tissue. (A) Representative image showing pancreatic progenitor cells expressing PDX-1 (red) and NKX6.1 (green). Yellow staining indicates co-expression of both markers in the majority of cells as is observed in the developing human pancreas. (B) Cells transplanted into mice can mature into endocrine and exocrine cells. Here endocrine clusters expressing synaptophysin (red) are surrounded by ductal structures expressing CK-19 (green). Data in (B) are from the laboratory of Dr. Timothy J. Kieffer (University of British Columbia, Vancouver, Canada).

Leukemia Research

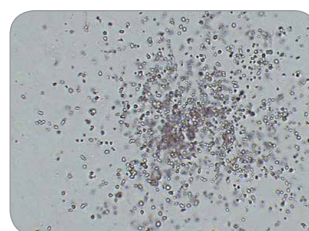
Leukemic cells have the capacity for clonogenic growth *in vitro*.⁵³ Often, culture methods and media used for the study of normal hematopoiesis are also useful for functional studies of leukemic cells. Leukemic cells can be cultured in colony-forming unit (CFU) assays in MethoCult™ medium, long-term culture-initiating cell (LTC-IC) assays in MyeloCult™ medium or in serum-free conditions with StemSpan™ serum-free expansion medium. Applications include research into the mechanisms underlying malignant transformation and cancer progression, or evaluating the responsiveness of patient cells to chemotherapeutic agents, such as specific inhibitors of the BCR-ABL tyrosine kinase in Chronic Myeloid Leukemia (CML).⁵⁴

Products for the Assay and Culture of Hematopoietic Stem and Progenitor Cells

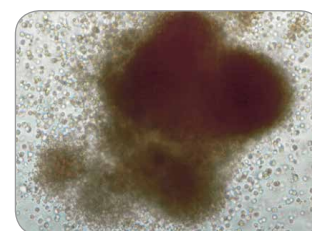
PRODUCT	APPLICATION	CATALOG #
MethoCult™ for Human Cells	Base media allowing for the addition of desired growth factors	04100 04230 04236 04330
	Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in bone marrow and blood	04034 04434 04435 04436
	Detection of CFU-GM (including CFU-G and CFU-M) in bone marrow and blood	04035 04534 04535 04536
MethoCult™ for Mouse Cells	Detection of BFU-E, CFU-GM and CFU-GEMM in bone marrow, spleen, peripheral blood and fetal liver	03434
	Detection of CFU-GM in bone marrow, spleen, peripheral blood and fetal liver	03534
	Base media allowing for the addition of desired growth factors	03334 03234 03231
StemSpan™ Serum-Free Expansion Medium	Serum-free medium for culture of hematopoietic cells	09600 09605 09800 09805 09900 09955
MyeloCult™	Myeloid long-term culture medium for primitive hematopoietic cells	05100 05300
UM729	Small molecule that enhances the self-renewal of human hematopoietic stem cells <i>in vitro</i>	72332

Hematopoietic Colony Images

Examples of Colonies Derived From Human Hematopoietic Progenitor Cells



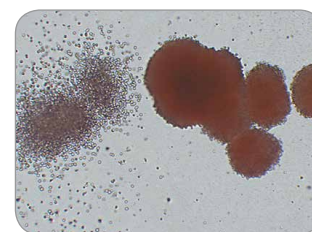
Human CFU-GM



Human CFU-GEMM

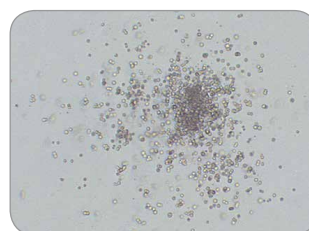


Human BFU-E

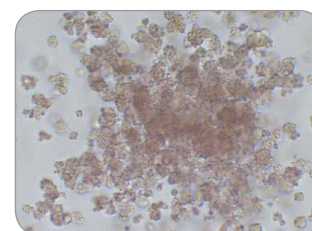


Human CFU-GM & BFU-E

Examples of Colonies Derived From Mouse Hematopoietic Progenitor Cells



Mouse CFU-M



Mouse BFU-E



WALLCHART

Human Hematopoietic Progenitors

www.stemcell.com/HumanHemaWallchart

T Cell Therapy Research

Take your T cell immunotherapy research from bench to bedside. STEMCELL Technologies has entered a collaboration with GE Healthcare to develop T cell isolation, activation and expansion reagents for commercial-scale cell therapy production. This collaboration aims to give researchers the confidence of a path to the clinic with cGMP-grade T cell reagents. Perform your preclinical research and optimize your protocols with these currently available research use only (RUO) versions developed by STEMCELL Technologies. Learn more at www.stemcell.com/t-cell-therapy.

Reagents for T Cell Therapy Research

PRODUCT	CATALOG #	APPLICATION
EasySep™ Release Human CD3 Positive Selection Kit	17751	Fast and easy isolation of CD3 ⁺ cells free of magnetic particles in under 30 minutes <ul style="list-style-type: none"> • HIGH PURITY. Achieve purities of up to 99% with high recoveries. • COLUMN-FREE. With no columns, EasySep™ is gentle on your cells.
ImmunoCult™ Human CD3/CD28 T Cell Activator	10971	Soluble reagents for T cell activation and expansion without beads <ul style="list-style-type: none"> • FLEXIBLE. Soluble and can be easily washed away. • GENTLE. Provides gentle stimulus that maintains high cell viability. • COMMERCIALLY AVAILABLE. Not exclusively licensed for the manufacturing of genetically modified T cells.
ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator	10970	
ImmunoCult™-XF T Cell Expansion Medium	10981	Serum- and xeno-free culture medium for T cell expansion <ul style="list-style-type: none"> • DEFINED FORMULATION. No need to supplement with serum. • OPTIMIZED. Formulated for rapid T cell expansion. • MAINTAIN T CELL PHENOTYPE. Similar proportions of CD4/CD8 cells to the start of culture.

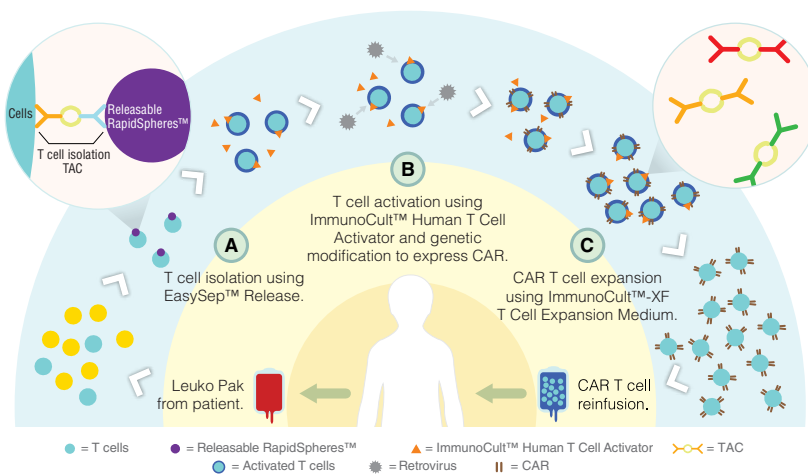


Figure 6. Integrated workflow for the manufacturing of chimeric antigen receptor (CAR) T cells using STEMCELL's products

Isolation of T cells using (A) EasySep™ Release CD3 Positive Selection Kit; T cell activation using soluble (B) ImmunoCult™ Human T Cell Activator and gene modification to express the CAR construct; and expansion of CAR T cells in xeno-free and serum-free (C) ImmunoCult™-XF T Cell Expansion Medium.

Cell Isolation for Cancer Research

Isolate cancer cells with our innovative cell separation platforms, RosetteSep™ and EasySep™, which provide an easy, fast and effective method for isolating rare cells with high purity and recovery.⁵⁵⁻⁵⁷ With RosetteSep™, cells are isolated directly from human whole blood during density gradient centrifugation, reducing your cell isolation workflow to a single step. With EasySep™, human cells are isolated immunomagnetically by either positive or negative selection from many types of samples without the use of columns. EasySep™ can be fully automated using RoboSep™.

APPLICATION	PRODUCT	CATALOG #	RECOMMENDED FOR:
Circulating Tumor Cells (CTCs)	RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD36	15127	Enrichment of CTCs directly by depleting hematopoietic cells from whole blood (WB). CD36 has been shown to be expressed on a small subset of breast cancer samples. ^{58,59} For enrichment of CTCs from breast cancer samples we recommend using #15122 or #15137.
		15167 (5 x 15127)	
	RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD56	15137	Enrichment of CTCs by depleting hematopoietic cells from WB. CD56 has been shown to be expressed on small cell lung cancer (SCLC) and pancreatic carcinoma samples. ⁶⁰⁻⁶² For enrichment of CTCs from SCLC and pancreatic carcinoma samples we recommend using #15122 or #15127.
		15177 (5 x 15137)	
	EasySep™ Direct Human CTC Enrichment Kit	19657	Enrichment of CTCs directly from WB without the need for pre-processing steps such as density gradient centrifugation, sedimentation or lysis.
CD45 Depletion	RosetteSep™ Human CD45 Depletion Kit	15122	Enrichment of CTCs by depleting CD45 ⁺ cells from WB.
		15162 (5 x 15122)	
	EasySep™ Human CD45 Depletion Kit*	18259 (PBMC)	The enrichment of CTCs by depleting CD45 ⁺ cells from fresh or previously frozen peripheral blood human mononuclear cells (PBMCs) or WB.
18289 (WB)			
Multiple Myeloma (CD138) Cells	RosetteSep™ Multiple Myeloma Cell Enrichment Cocktail	15129	Enrichment of untouched multiple myeloma cells (B cells and plasma cells) from bone marrow aspirates.
		15169 (5 x 15129)	
	EasySep™ Human CD138 Positive Selection Kit*	18357 (PBMC)	Selection of highly purified CD138 ⁺ cells from PBMCs, bone marrow (BM) or WB.
		18387 (BM and WB)	
B Cells From Chronic Lymphocytic Leukemia (CLL) Samples	EasySep™ Human B Cell Enrichment Kit without CD43 Depletion*	19154	Enrichment of untouched B cells from PBMCs of leukemia or lymphoma samples, in which B cells may express CD43.
	EasySep™ Direct Human B-CLL Cell Isolation Kit	19664	Enrichment of untouched B cells from whole blood of CLL samples, in which B cells may express CD43. Cells are enriched without the need for density gradient centrifugation, sedimentation or lysis.

*Automate EasySep™ cell isolations with RoboSep™ instruments (www.RoboSep.com)

Small Molecules

Small molecules can be used in cancer research to understand mechanisms of cancer, identify signaling pathways, assess the effect of inhibiting certain signals, and more. Small molecules may also be tested in vitro as potential therapeutics. A wide range of small molecules are available from STEMCELL Technologies that are used in high impact cancer research. For a complete listing of the small molecules available, please visit www.stemcell.com/smallmolecules.

Contract Assay Services



STEMCELL Technologies' Contract Assay Services works with you to design and execute customized cell-based assays to meet your needs. Our primary cell-based assays can provide clinically relevant results of the effects of small molecule compounds, including chemotherapeutic agents, or biologics on your cell type of interest. These assays can also assess effects on the proliferation and differentiation of hematopoietic or mesenchymal stem and progenitor cells, as well as various immune cells. Join the more than 120 organizations worldwide that have trusted our experts to conduct more than 600 studies to answer their questions and achieve their goals.

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Brain Tumor Stem Cells

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