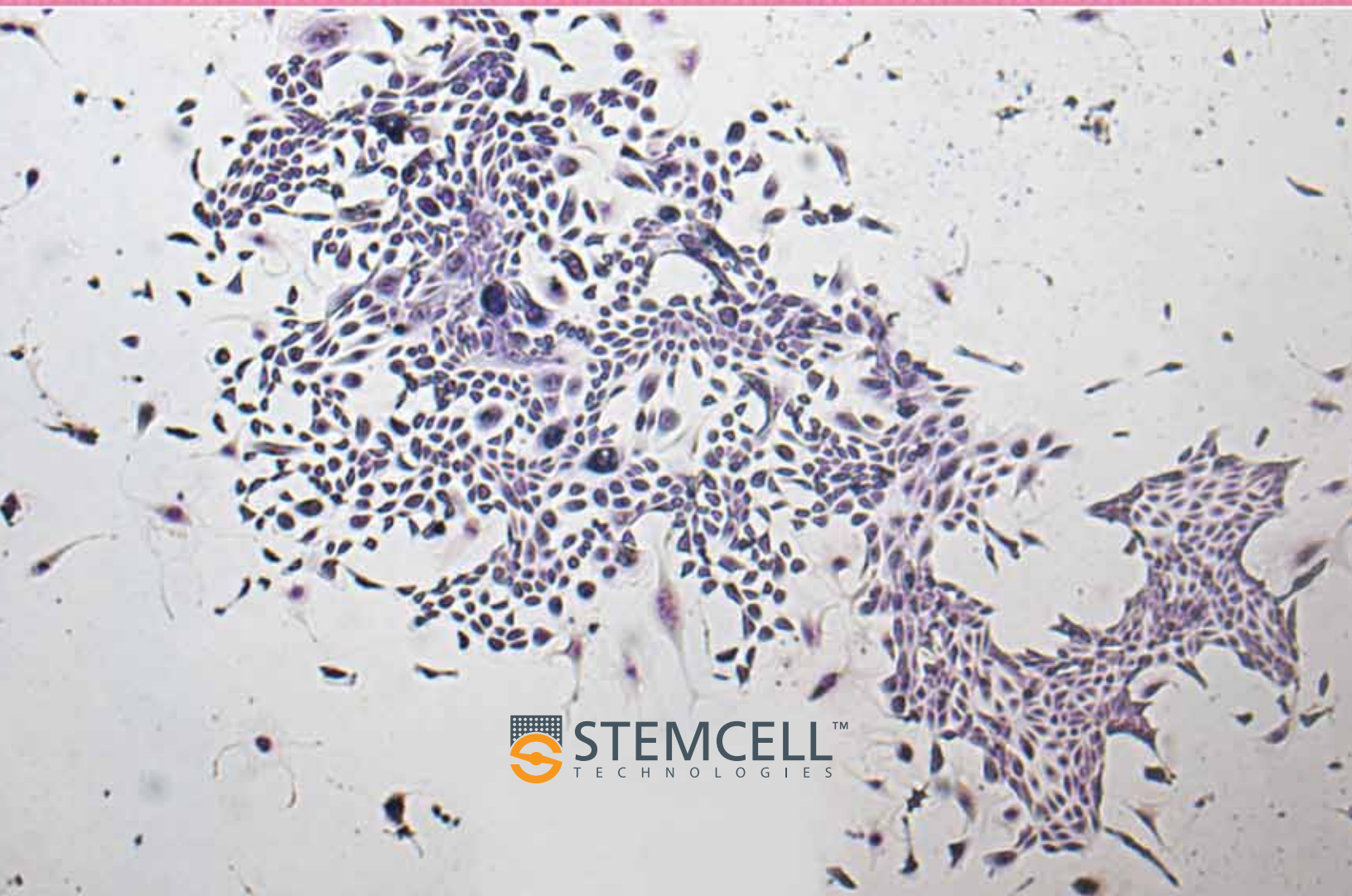
Mammary Epithelial Cells

Standardized Media and Reagents



STEMCELL™ TECHNOLOGIES

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Serving Mammary Epithelial Cell Research Scientists

STEMCELL Technologies, a privately-owned biotechnology company, is a leader in specialty cell culture media, cell separation products and ancillary reagents for life science research. Driven by science, STEMCELL delivers over 1500 products to more than 70 countries worldwide. To learn more about how STEMCELL helps make research work, visit www.stemcell.com.

Front cover: Human mammary bipotent epithelial cell colony cultured in EpiCult™-B (Human) and visualized with Wright-Giemsa. Photo courtesy of Dr. C. Eaves and P. Eirew.

Mammary Epithelial Cell Research
Cell Types and Assays

Emerging evidence suggests that the mammary epithelium in both humans and mice may comprise a hierarchy of cells, spanning from mammary stem cells to differentiated luminal and myoepithelial cells (Figure 1).

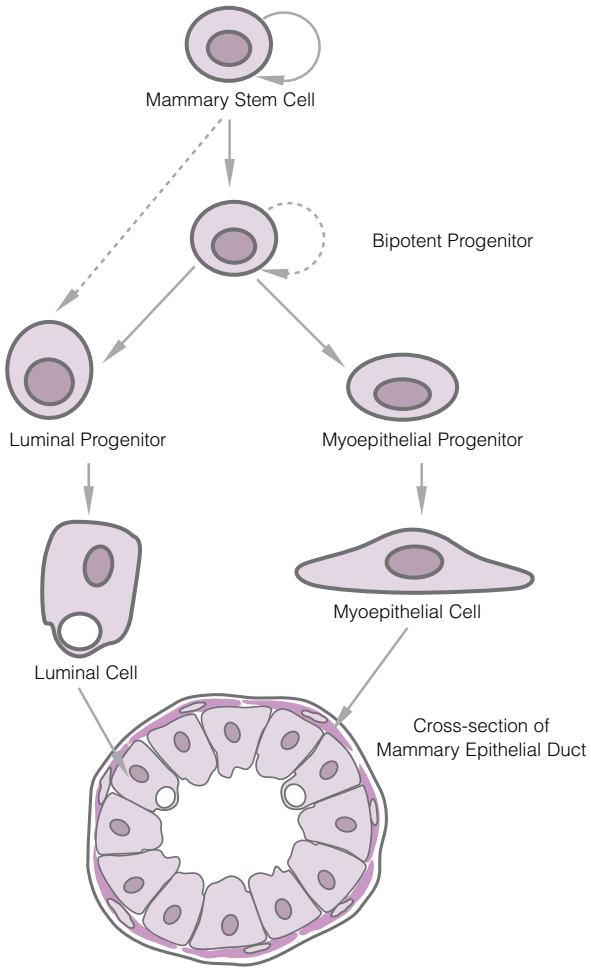
In the gold standard in vivo assay for mammary stem cells, multi-lineage outgrowths are generated in primary and secondary recipient mice from limiting numbers of cells. Self-renewing cells that generate such multi-lineage outgrowths are termed mammary repopulating units (MRUs).

Mammary progenitor cells can be detected by their ability to generate colonies in vitro, either in liquid culture on tissue culture plastic or within 3-dimensional gels such as BD Matrigel™ (pg. 9). These cells are termed mammary colony-forming cells (Ma-CFCs).

Primitive cells within the mammary epithelium can also be detected with an alternate in vitro assay: the mammosphere assay (pg. 11). Some mammosphere-initiating cells are perceived to be highly primitive precursor cells since clonal mammospheres can form secondary mammospheres and generate multiple lineages of daughter cells.

Support your work with these assays and cell types by leveraging premium, standardized mammary cell products from STEMCELL Technologies (www.stemcell.com).

FIGURE 1. Mammary Epithelial Cell Hierarchy



Why Choose Products From STEMCELL Technologies?

COMPREHENSIVE. Culture, characterize and differentiate primary human and mouse mammary epithelial cells with products based on strong research expertise and specialized technical support.

CONSISTENT. Maximize the reproducibility of your experimental results with fully defined and rigorously tested culture media.

CONVENIENT. Save significant time and effort and minimize experiment troubleshooting with our easy-to-use products and pre-tested protocols, optimized for specific applications.

Mammary Epithelial Cell Research

Product Overview

Mammary Tissue Dissociation	Dissociation Enzymes (pg.5)
Viable Precursor Cell Detection	ALDEFLUOR™ Enzymatic Assay for Detection of ALDH^{br} cells (pg.6)
Cell Isolation or Enrichment	EasySep™ Cell Separation (pg.7)
Functional In Vitro Assays	EpiCult™-B for Ma-CFC and 3D Morphogenesis Assays (Human pg.9; Mouse pg.13)
Standardized Culture Media	EpiCult™-C for Short-Term Culture (Human; pg.10) MammoCult™ for Mammospheres (Human; pg.12) EpiCult™-B for Short-Term Culture (Mouse; pg.13)

WEBINAR

Dr. John Stingl: Assays for Mammary Epithelial Stem and Progenitor Cells

www.stemcell.com/StinglAssaysWebinar

SCAN ME

WALLCHART

Assays for Human Mammary Stem and Progenitor Cells

www.stemcell.com/MammaryPoster

SCAN ME

Dissociation Enzymes

Dissociate Mammary Gland Tissues

Although different techniques are required for mouse and human mammary tissue dissociation, the samples from both species can be effectively dissociated into a single cell suspension containing viable mammary stem or progenitor cells.

Enzymatic dissociation of mouse mammary organoids usually occurs after 6-8 hours of incubation with a collagenase-hyaluronidase cocktail, which can be extended overnight using our new, gentler cocktail. The tissues are further dissociated into a single cell suspension using Trypsin-EDTA, DNase I and Dispase, and the resulting cell suspension is filtered through a 40 µm cell strainer to remove clumps. Human breast samples are similarly dissociated with collagenase/hyaluronidase enzymes (standard strength) overnight. The human mammary organoids, obtained through differential centrifugation, can then be dissociated into a single cell suspension using Trypsin/EDTA, Dispase and DNase enzymes.

NEW: Overnight Tissue Dissociation

With the new Gentle Collagenase/Hyaluronidase cocktail that dissociates mouse mammary gland tissues in 12 hours (rather than the usual 6-8), experiments can be completed the next day rather than late at night.

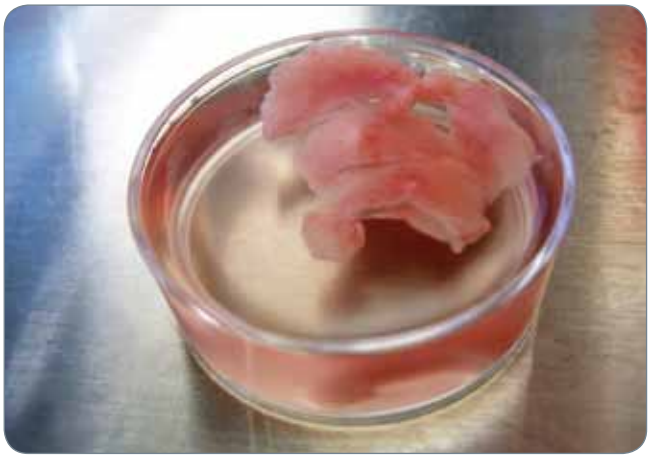
Why Optimize Tissue Dissociation?

Following an optimized dissociation protocol and using quality dissociation enzymes will yield higher cell numbers and increased cell viability. Download a guide to the dissociation of solid mammary tissue at www.stemcell.com.

To Dissociate Mammary Gland Tissue

PRODUCT	QUANTITY	CATALOG #
Collagenase/Hyaluronidase (10X)	10 mL	07912
Gentle Collagenase/Hyaluronidase (10X)	10 mL	07919 NEW!
Trypsin-EDTA	500 mL	07901
DNase I (1 mg/mL)	1 mL	07900
Dispase (5 mg/mL)	100 mL	07913
40 µm Cell Strainer	50/case	27305

FIGURE 2. Mammary Tissues



Freshly extracted mouse mammary glands ready for enzymatic dissociation to a single cell suspension.

A human reduction mammoplasty sample.

ALDEFLUOR™

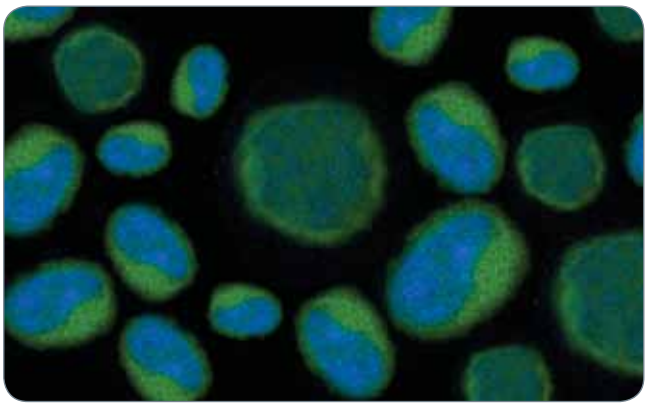
Detect Viable Mammary Precursor Cells

ALDEFLUOR™ detects both primitive mammary cells and breast cancer stem cells, based on their expression of aldehyde dehydrogenase (ALDH). Although this fluorescent assay was first developed for hematopoietic stem cells, optimized protocols for assaying some normal and malignant mammary cells with ALDEFLUOR™ are available from techsupport@stemcell.com.

Why Choose ALDEFLUOR™?

- The only non-immunological assay for detecting primitive mammary cells
- Non-destructive, allowing for the downstream analysis of ALDH^{br} cells.
- Compatible with immunophenotyping and counterstaining of cells with other fluorescent antibodies.

FIGURE 3. SKBR3 breast cancer cells visualized with ALDEFLUOR™



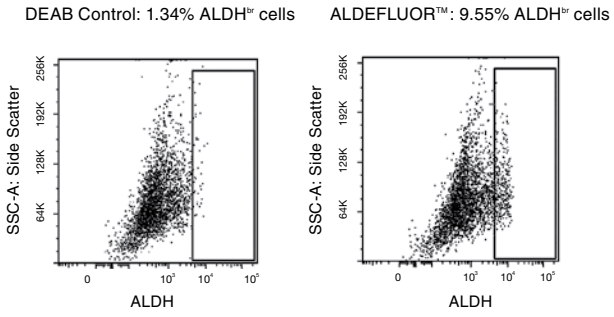
To Detect Viable Precursor Cells

PRODUCT: ALDEFLUOR™ Kit
CATALOG #: 01700 40 Tests/Kit

RECOMMENDED FOR:
Identification, enumeration and isolation of viable normal and cancer stem/progenitor cells based on their ALDH activity.

CONTAINS:
- ALDEFLUOR™ (Dry reagent, 50 µg)
- DEAB (1.5 mM Diethylaminobenzaldehyde in 95% ethanol, 1 mL)
- HCl (2N Hydrochloric Acid, 1.5 mL)
- DMSO (Dimethylsulphoxide, 1.5 mL)
- ALDEFLUOR™ Assay Buffer (4 x 25 mL)

FACS Profile Results With ALDEFLUOR™ Kit



Starting with primary human mammary epithelial cells, ALDH^{br} cell content of the selected cells typically ranges around 10%.

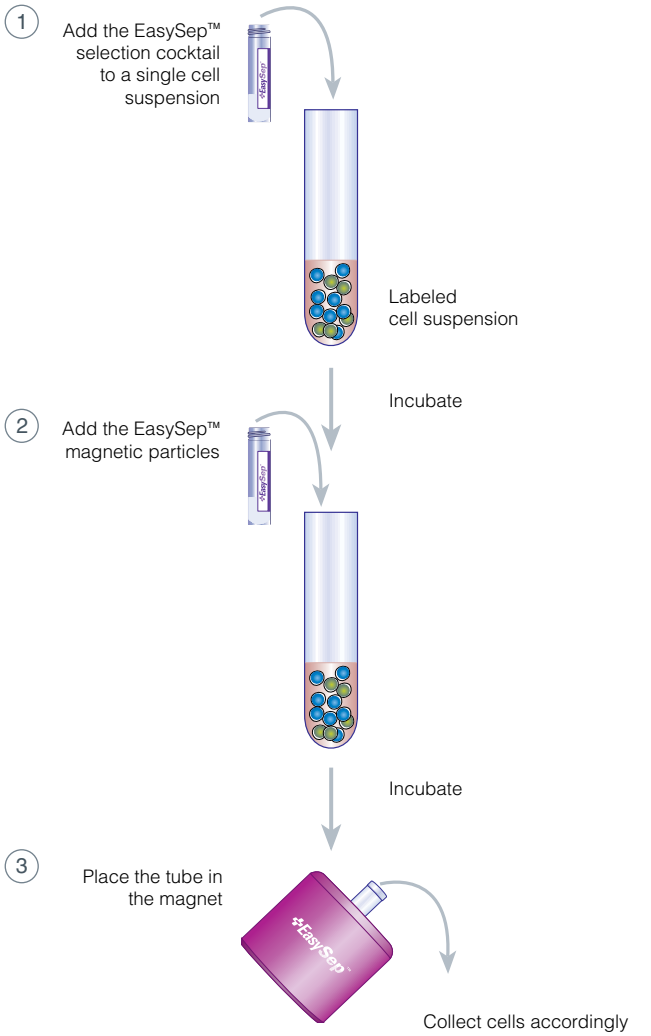
Technical Bulletin: ALDEFLUOR™ and Cancer Stem Cells

Obtain a technical bulletin detailing publications, technical data and protocols on using the ALDEFLUOR™ assay with breast cancer cell lines at www.stemcell.com.

EasySep™

Isolate Human Mammary Epithelial Cells

FIGURE 4. Schematic of EasySep™ immunomagnetic cell isolation.

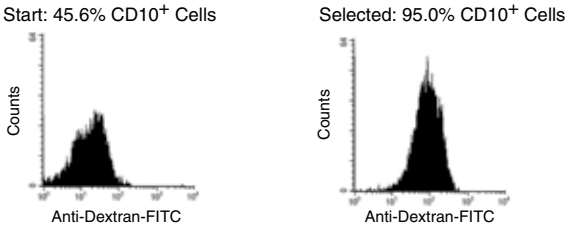


Investigating Circulating Tumor Cells (CTCs)?

Try the RosetteSep™ Human Circulating Epithelial Tumor Cell Enrichment Cocktail (Catalog #15127).

To Isolate Human CD10⁺ Cells

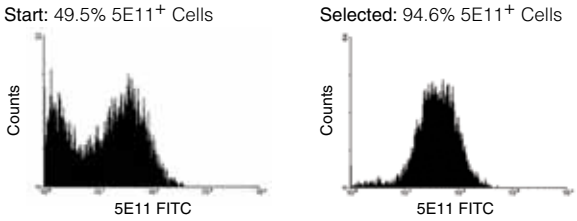
EasySep™ Human CD10 Positive Selection Kit (Catalog #18358) is optimized for the isolation of CD10⁺ cells; the CD10⁺ cell fraction contains bipotent and myoepithelial-restricted progenitors, as well as non-clonogenic myoepithelial cells.



The CD10⁺ cell content of the enriched fraction typically ranges from 84 - 98%. The purity of the CD10⁺ cells can be assessed with FITC-labeled anti-dextran (which recognizes the dextran on the magnetic particle).

To Isolate Human EpCAM⁺ Cells

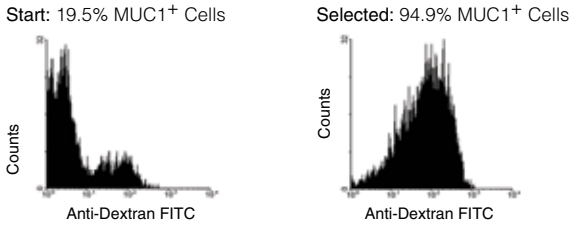
EasySep™ Human EpCAM Positive Selection Kit (Catalog #18356) is optimized for the isolation of EpCAM⁺ cells; the EpCAM⁺ fraction contains bipotent and luminal-restricted progenitors, as well as luminal cells.



Starting with cultured mammary tissue, the typical purity of the selected fraction is 90 - 96%. Purity has been assessed by staining with the monoclonal antibody 5E11-FITC (Catalog #10110) which has a distribution identical to EpCAM.

To Isolate Human MUC1⁺ Cells

EasySep™ Human MUC1 Positive Selection Kit (Catalog #18359) is optimized for the isolation of MUC1⁺ cells; the MUC1⁺ fraction contains luminal-restricted progenitors as well as non-clonogenic luminal epithelial cells.



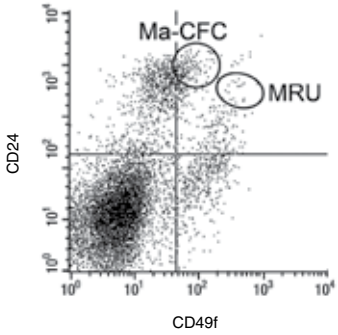
Starting with cultured mammary tissue, the typical purity of the enriched fraction is 95 - 99%. The purity of MUC1⁺ cells can be assessed with FITC-labeled anti-dextran (which recognizes the dextran on the magnetic particle).

EasySep™

Isolate Mouse Mammary Epithelial Cells

To Enrich For Mouse Mammary Stem Cells

EasySep™ Mouse Mammary Stem Cell Enrichment Kit (Catalog #19757) is optimized for the isolation of $CD31^-CD45^-TER119^-$ $CD24^+CD49f^{++}$ population; the sub-population of $CD24^+CD49f^{++}$ is enriched for mammary epithelial stem cells and is isolated via fluorescence-activated cell sorting.

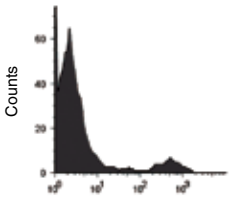


Flow cytometry profile of freshly dissociated mouse mammary epithelial cells distributed according to their expression of CD49f and CD24. The mammary repopulating unit (MRU)-rich fraction is found in the $CD49f^{++}CD24^+$ subset. The mammary colony-forming cells (Ma-CFCs) are found in the $CD49f^+CD24^{++}$ subset.[2]

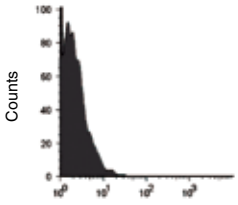
To Enrich For Mouse Epithelial Cells

EasySep™ Mouse Epithelial Cell Enrichment Kit (Catalog #19758) removes contaminating hematopoietic and endothelial cells from cell preparations. Cells expressing CD31, CD45 and TER119 cell surface antigens are depleted, leaving behind an unlabeled epithelial cell-enriched population.

Before Depletion:
10.37% ± 0.50% unwanted cells



After Depletion:
1.12% ± 0.22% unwanted cells



Purity has been assessed by staining with goat anti-rat IgG FITC, which recognizes the antibodies used to deplete unwanted cells expressing CD45, TER119 and/or CD31.

EasySep™

Mammary Epithelial Cell Isolation Kit Listing

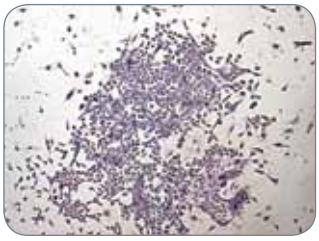
EASYSEP™ SELECTION KIT	SPECIES	COMPONENTS	CAPACITY	CATALOG #
CD10 Positive Selection	Human	EasySep™ Human CD10 Positive Selection Cocktail EasySep™ Magnetic Particles	1 x10^9 cells	18358
EpCAM Positive Selection	Human	EasySep™ Human EpCAM Positive Selection Cocktail EasySep™ Magnetic Particles	1 x10^9 cells	18356
MUC1 Positive Selection	Human	EasySep™ Human MUC1 Positive Selection Cocktail EasySep™ Magnetic Particles	1 x10^9 cells	18359
Mammary Stem Cell Enrichment	Mouse	EasySep™ Mouse Mammary Epithelial Cell Enrichment Cocktail EasySep™ Biotin Selection Cocktail EasySep™ Magnetic Particles Anti-CD24-PE, Anti-CD49f-FITC	1 x10^9 cells	19757
Epithelial Cell Enrichment	Mouse	EasySep™ Mouse Epithelial Cell Enrichment Cocktail EasySep™ Biotin Selection Cocktail EasySep™ Magnetic Particles	1 x10^9 cells	19758

EpiCult™-B (Human)

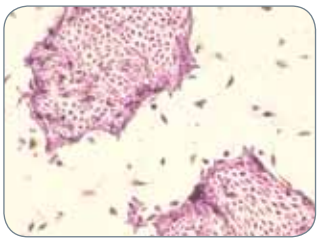
Assay For Human Mammary Epithelial Cells

EpiCult™-B is a versatile medium that can assay for the presence of human mammary colony-forming cells (Ma-CFCs) in either adherent monolayer or in 3D Matrigel™-based cultures. Quantifying Ma-CFC frequency provides a functional measure of the number of mammary progenitor cells in the sample, while the 3D morphogenesis assay enables more representative studies of the in vivo mammary duct structure.

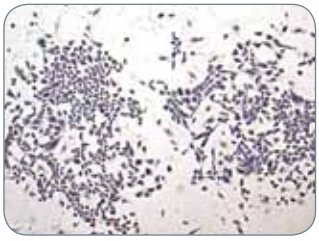
The Ma-CFC Assay



Mixed Epithelial Colony



Luminal Epithelial Colony



Myoepithelial Colony

The 3D Morphogenesis Assay

3D Mammary Structure

Why Choose EpiCult™-B (Human)?

- Fully defined culture medium
- Promotes clonogenic growth of unipotent and bipotent progenitors in the Ma-CFC assay
- Promotes mammary progenitor cell differentiation in the 3D morphogenesis assay.

To Assay For Mammary Colony-Forming Cells

PRODUCT:	EpiCult™-B (Human)
CATALOG #:	05601 500 mL

RECOMMENDED FOR:

Culture of human mammary luminal and myoepithelial cells. Promotes the clonogenic growth of bipotent, luminal-restricted and myoepithelial-restricted human mammary epithelial progenitors when used in conjunction with an irradiated feeder layer (NIH 3T3). Also used for the enzymatic dissociation of human mammary tissue when supplemented with Collagenase/ Hyaluronidase (Catalog #07912).

CONTAINS:

- EpiCult™-B Basal Medium (Human)
- EpiCult™-B Supplement (Human)

REQUIRES:

Supplement with 0.48 µg/mL freshly dissolved Hydrocortisone (Catalog #07904) before use.

EpiCult™-C (Human)

Culture Human Mammary Epithelial Cells

EpiCult™-C is a serum-free liquid culture medium optimized for the short term culture of primary human mammary epithelial cells as adherent monolayers (Figure 5). It provides balanced, robust growth of both myoepithelial and luminal cells.

Why Choose EpiCult™-C?

- Fully defined culture medium
- Promotes robust growth of luminal and myoepithelial cells
- Uniquely optimized for the short-term culture of primary mammary epithelial cells

To Culture Human Mammary Epithelial Cells

PRODUCT: EpiCult™-C
CATALOG #: 05630 500 mL

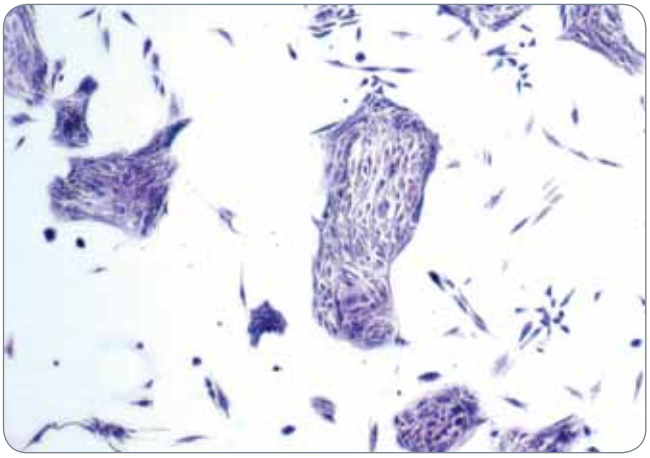
NEW!

RECOMMENDED FOR:
Routine monolayer culture of primary human mammary epithelial cells and a variety of breast cancer cell lines.

CONTAINS:
- EpiCult™-C Basal Medium (Human)
- EpiCult™-C Proliferation Supplements (Human)

REQUIRES:
Supplement with 0.48 µg/mL freshly dissolved Hydrocortisone (Catalog #07904) before use.

FIGURE 5. EpiCult™-C 6-Day Culture



Mammary human epithelial cells cultured in EpiCult™-C for 6 days and visualized with Wright-Giemsa.

VIDEO

Introducing EpiCult™-C Medium

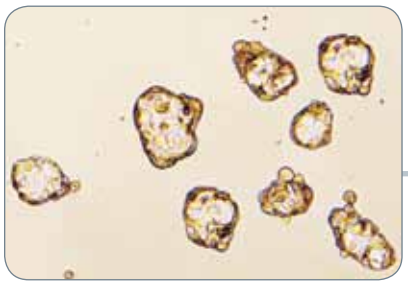
www.stemcell.com/EpiCultCVideo

SCAN ME

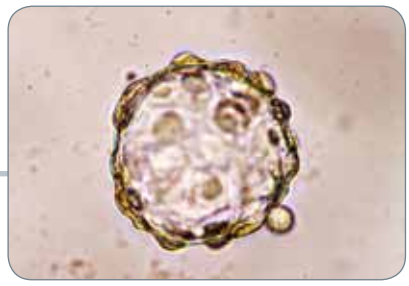
MammoCult™

Culture Human Mammospheres or Tumorspheres

Mammosphere culture involves seeding mammary epithelial cells at low density in an environment that prevents their adherence to a substratum and enables their proliferation in suspension as spherical clusters. Primitive mammary cells have been detected over multiple generations of mammospheres, and cells from dissociated mammospheres demonstrate multi-lineage differentiation potential in the Ma-CFC assay. The mammosphere culture system is thus capable of propagating mammary stem and progenitor cells in vitro.



Mammospheres can be classified into different groups based on morphology.

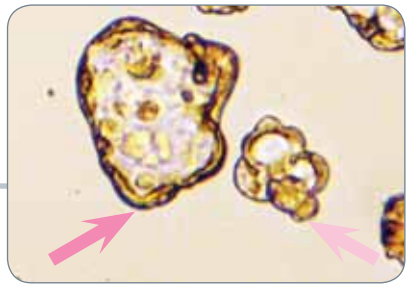


Hollow Mammosphere

This hollow mammosphere was cultured in suspension in MammoCult™ for 7 days.

Solid Mammospheres

These solid mammospheres were cultured in suspension in MammoCult™ for 7 days.



Large and Small Mammospheres

These large (>70 µm) and small (< 70 µm) mammospheres were cultured in suspension in MammoCult™ for 7 days.

Protocol: Tumorsphere Culture

Cells from breast cancer cell lines may also be grown in nonadherent liquid suspension cultures. Obtain a technical bulletin on tumorsphere culture with detailed protocols at **www.stemcell.com**.

MammoCult™

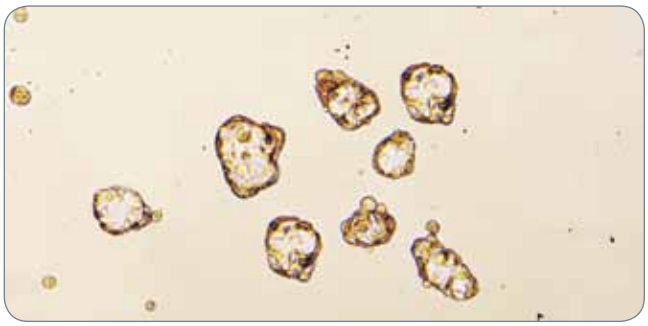
Culture Human Mammospheres or Tumorspheres

MammoCult™ is the only commercially available medium for the culture of mammospheres and tumorspheres (Figures 6 and 7). It supports robust cultures that produce significantly larger numbers of mammospheres and tumorspheres than published formulations. Use this defined culture medium to maintain primary mammary epithelial progenitors or breast cancer cell lines.

Why Choose MammoCult™?

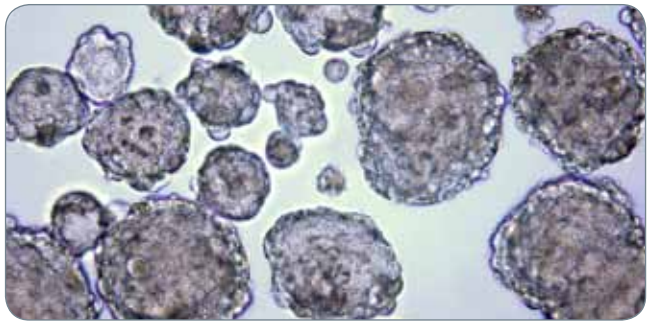
- Fully defined culture medium
- Generates a large number of robust mammospheres and tumorspheres
- Supports short-term maintenance of primary mammary epithelial progenitors
- Supports long-term maintenance of breast cancer cell lines MCF7, SKBR3, MDA -MB-231, AU 565, SUM149 and BT474.

FIGURE 6. MammoCult™ Mammosphere Culture



Mammospheres derived from primary mammary tissue and cultured in liquid suspension in MammoCult™ for 8 days.

FIGURE 7. MammoCult™ Tumorsphere Culture



Tumorspheres derived from the MCF-7 breast cancer cell line and cultured in liquid suspension in MammoCult™ for 8 days.

To Culture Mammospheres and Tumorspheres from Human Mammary Cells

PRODUCT:	MammoCult™
CATALOG #:	05620 500 mL

RECOMMENDED FOR:

Culture of primary human mammary stem and progenitor cells and breast cancer cell lines as mammospheres and tumorspheres.

CONTAINS:

- MammoCult™ Basal Medium (Human)
- MammoCult™ Proliferation Supplement (Human)

REQUIRES:

Supplement with 0.48 µg/mL freshly dissolved Hydrocortisone (Catalog #07904) and 4 µg/mL Heparin (Catalog #07980) before use.

MammoCult™ Scientific Poster

MammoCult™ outperforms the published formulation (Dontu et al. 2003), by maintaining a larger number of mammospheres and preserving both unipotent and bipotent progenitor cells longer.

Download the scientific poster under Technical Resources at www.stemcell.com.

EpiCult™-B (Mouse)

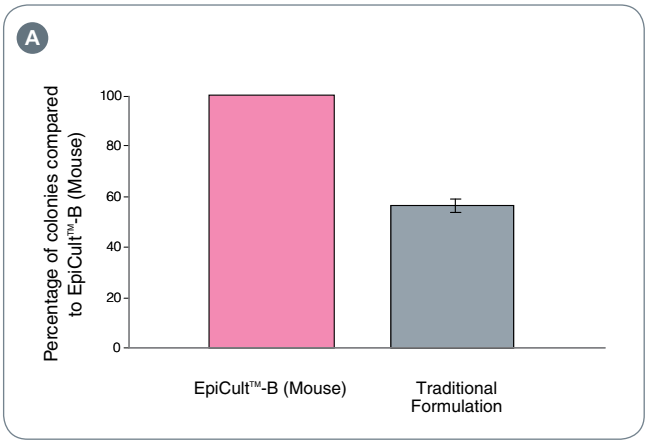
Culture and Assay Of Mouse Mammary Epithelial Cells

EpiCult™-B (Mouse) is a robust culture medium for mouse mammary epithelial cells (Figure 8), and is ideal for assaying the presence of mouse mammary colony-forming cells (Ma-CFCs) in either adherent monolayer or in 3D Matrigel™-based cultures. As this medium is formulated specifically for mouse cells, it delays the epithelial-to-mesenchymal transition (EMT) commonly observed in the mouse mammary epithelial in vitro cultures (Figure 9).

Why Use EpiCult™-B (Mouse)?

- Defined culture medium for primary mammary epithelial cells (short-term)
- Promotes clonogenic growth of mammary epithelial cells in the Ma-CFC assay
- Promotes mammary progenitor cell growth in the 3D morphogenesis assay.
- Delays and decreases EMT occurrence in cell cultures, compared to the traditional medium

FIGURE 8. EpiCult™-B Mouse Mammary Epithelial Cell Colonies



(A) EpiCult™-B (Mouse) generates twice as many colonies compared to commonly used formulation. (B) and (C) are examples of mouse mammary epithelial colonies grown in EpiCult™-B (Mouse).

To Assay For Mammary Colony-Forming Cells

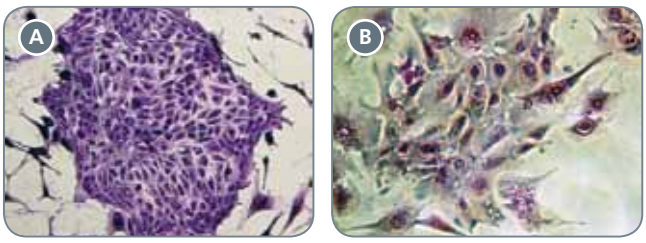
PRODUCT: EpiCult™-B (Mouse)
CATALOG #: 05610 500 mL

RECOMMENDED FOR:
Colony-forming cell assays with mouse mammary epithelial cells. Promotes the clonogenic growth of mouse mammary progenitors when used in conjuction with an irradiated feeder layer (NIH 3T3). It is also used for the enzymatic dissociation of mouse mammary tissue when supplemented with Collagenase/ Hyaluronidase (Catalog #07912).

CONTAINS:
- EpiCult™-B Basal Medium (Mouse)
- EpiCult™-B Proliferation Supplements (Mouse)

REQUIRES:
Supplement with 10 ng/mL rh Epidermal Growth Factor (Catalog #02633), 10 ng/mL rh Basic Fibroblast Growth Factor (Catalog #02634) and 4 µg/mL Heparin (Catalog #07980) before use.

FIGURE 9. EpiCult™-B (Mouse) Cell Cultures Display Decreased EMT



Mammary epithelial cells display a clear delay and decrease of epithelial-to-mesenchymal transition (EMT) when cultured in (A) EpiCult™-B(Mouse) versus (B) traditional formulation (photo courtesy of Dr. John Stingl). EMT is defined by emergence of a fibroblastic-like morphology.

Antibodies and Miscellaneous

Support Products

Antibodies

ANTIGEN	CLONE	EXPRESSION	ISOTYPE	FORMAT	APPLICATIONS*	QUANTITY	CATALOG #
CD10 (CALLA)	FR4D11	Basal-positioned cells	Mouse IgG_1	Purified	CS, IH	100 µg	01431
CD24	32D12	Luminal cells	Mouse IgG_1, κ Mouse IgG_1, κ Mouse IgG_1, κ	Purified Biotin FITC	CS CS CS	100 µg 100 tests 100 tests	01434 10231 10424
CD44	IM7	Human mammary tumor stem cells	Rat IgG_{2b}, κ Rat IgG_{2b}, κ Rat IgG_{2b}, κ	Purified Biotin FITC	CS CS CS	50 µg 500 µg 50 µg	01465 10232 10432
CD49f (α6 Integrin)	GoH3	Basal-positioned cells, human and mouse progenitors[1,3,4] and mouse stem cells	Rat IgG_{2a}	FITC	FC	100 tests	10111
CD90 (Thy1)	5E10	Myoepithelial progeny of human mammary epithelial cells	Mouse IgG_1, κ Mouse IgG_1, κ	Purified FITC	FC, CS FC, CS	100 µg 100 tests	01437 10427
EpCAM	VU-1D9	Bipotent and luminal-restricted progenitors and luminal cells	Mouse IgG_1 Mouse IgG_1	Purified FITC	CS, IH FC, IF	100 µg 100 tests	01420 10109
	323/A3	Same as VU-1D9 but 323/A3 has a slightly broader distribution in basal compartment	Mouse IgG_1	Purified	CS, IH	100 µg	01421
Epithelial Cell Surface	5E11	Luminal cells. Variable staining of malignant breast epithelium	Mouse IgG_1 Mouse IgG_1	Purified FITC	CS, IH FC	100 µg 100 tests	01422 10110
Keratin 18	DC-10	Luminal cells	Mouse IgG_1	Purified	IH	100 µg	01425
Keratin 19	A53-B/A2	Subpopulation of luminal cells	Mouse IgG_{2a}, κ	Purified	IH	100 µg	01432
Ly-6A/E (SCA1)	E13-161.7	Mouse mammary stem cells (weak) and some progenitors	Rat IgG_{2a}, κ Rat IgG_{2a}, κ	FITC PE	FC, CS FC, CS	500 µg 200 µg	10716 10816
MUC1 Glycoprotein	214D4	Luminal-restricted progenitors and luminal cells	Mouse IgG_1	Purified	CS, IH	200 µg	01423
Progesterone Receptor	SP2	Subpopulation of luminal cells	Rabbit IgG	Supernatant	IH	500 µg	01466

*** Applications:** CS - Cell Separation; FC - Flow Cytometry; IH – Immunochemistry IF - Immunofluorescence **Format:** FITC - Fluorescein Isothiocyanate; PE - Phycoerythrin

Support Products

PRODUCT	QUANTITY	CATALOG #
Collagen Solution (Bovine)	35 mL	04902
Hanks' Balanced Salt Solution Modified	500 mL	37150
Tissue Dissociation Flask (250 mL)	1 flask	27300
Tissue Culture Dishes (35 mm)	20 dishes/pack	27114
Ultra-Low Adherent Plates (6-well plates)	5 plates/pack	27145

Selected Publications

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11. Bardy P, et al., Isolation and Analysis of Different Subpopulation of Normal Human Breast Epithelial Cells after their Infection with a Retroviral Vector Encoding a Cell Surface Marker. Breast Cancer Res Treat 2:153-165, 1997
12. Claudio JA and Emerman JT. The Effects of Cyclosporin A, Tamoxifen and Medroxyprogesterone acetate on the Enhancement of Adriamycin Cytotoxicity in Primary Cultures of Human Breast Epithelial Cells. Breast Cancer Res Treat 2:111-112, 1996
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