

# TECHNICAL BULLETIN

## MOUSE MAMMARY STEM CELL ISOLATION

### ISOLATION AND CHARACTERIZATION OF MOUSE MAMMARY STEM CELLS

#### INTRODUCTION

Mammary stem cells sustain the supply of mammary epithelial cells throughout the lifetime of an organism. In the simplified scenario, this occurs in a hierarchical fashion, where stem cells give rise to more differentiated multilineage progenitors, which in turn generate lineage-restricted progeny. Overwhelming evidence suggests a direct link between normal and cancer stem cells and understanding the processes involved in normal mammary development and differentiation will likely provide insight into how deregulation leading to malignant transformation takes place.

Recent advances in immunomagnetic cell separation and multi-parameter cell sorting, as well as *in vitro* and *in vivo* functional assays, have permitted the study of mammary stem and progenitor cells. For example, in a paper recently published in *Nature*, Stingl *et al.* used these techniques to isolate and characterize cell populations enriched for mammary progenitors and stem cells.<sup>1</sup>

STEMCELL Technologies offers products for the enrichment and *in vitro* analysis of mammary stem and progenitor cells based on the methodology developed by Stingl *et al.*<sup>1</sup> Enzymes (including **Collagenase/Hyaluronidase**, **Dispase** and **DNase I**) and protocols are available for the efficient dissociation of mammary tissue into a single cell suspension. The **EasySep<sup>®</sup> Mouse Mammary Stem Cell Enrichment Kit** has been designed for the isolation of enriched fractions of mammary stem and progenitor cells from mouse tissue. Optimally formulated **EpiCult<sup>®</sup>-B (Mouse) Medium** supports the proliferation of mouse mammary epithelial cells. Together, the EpiCult<sup>®</sup> product range for mammary cells enables the study of processes that regulate normal mammary stem cell and progenitor behavior.

#### FUNCTIONAL CHARACTERIZATION OF MAMMARY STEM AND PROGENITOR CELLS

**Mammary stem cells** are functionally defined as those cells that can self renew and can generate ductal lobular outgrowths *in vivo* complete with both luminal and myoepithelial cells. These cells are operationally referred to as mammary repopulating units (MRUs) and can be detected using an *in vivo* "Fat Pad" assay.<sup>1,2</sup> Mammary epithelium in weaning mice is localized in a small segment of the mammary fat pad that, when removed, leaves behind a "cleared fat pad". Upon injection into this cleared fat pad, a single stem cell has the ability to regenerate histologically normal mammary outgrowths within 3 - 6 weeks.<sup>1,2</sup> In studies conducted by Stingl *et al.*<sup>1</sup> and Shackleton *et al.*<sup>2</sup> this technique, in combination with a limiting dilution analysis, indicated the presence of over 1400 MRUs per inguinal mouse mammary gland with a frequency of 1 per 1,400 mammary cells. The purification of stem cells from mouse mammary epithelial tissues is possible using specific dissociation procedures in combination with EasySep<sup>®</sup> immunomagnetic cell separation.

**Mammary progenitor cells** (or mammary colony-forming cells; Ma-CFCs) can be quantified using a colony-forming cell assay. In this functional assay, Ma-CFCs are grown with EpiCult<sup>®</sup>-B (Mouse) medium and Fetal Bovine Serum (FBS) on a layer of subconfluent irradiated NIH 3T3 fibroblasts (feeders). After a week in culture, Ma-CFCs develop into discrete adherent colonies that can be further analyzed using immunocytochemistry for the presence of epithelial-specific markers (i.e. keratins 14, 18, and 19). Using this functional assay, Stingl *et al.* reported the presence of over 30,000 Ma-CFCs in a mouse mammary gland, with a frequency of 1 per 63 dissociated mammary gland cells.<sup>1</sup>

Cells from both mammary progenitor and mammary stem cell-enriched fractions can also be grown in 3D cultures containing collagen or reconstituted basement membrane such as Matrigel<sup>™</sup>. In this system, two types of colonies develop. One type resembles ductal-alveolar structures found *in vivo*, and are believed to originate from early progenitor cells, if not stem cells. The other colonies are hollow, acinar-like structures composed of cuboidal epithelium.

#### STEM AND PROGENITOR CELL ENRICHMENT

Isolation of a mammary epithelial stem cell-enriched fraction has traditionally been difficult and time consuming due to the rarity of stem cells in the mammary gland. Mouse mammary stem cell (MRU)-enriched fractions have a reported phenotype of CD49f<sup>++</sup>CD24<sup>+</sup>,<sup>1,2\*</sup> whereas progenitor cells (Ma-CFCs), which are deficient in their ability to engraft clear fat pads, have a CD49<sup>+</sup>CD24<sup>++</sup> phenotype.<sup>1,2</sup> To simplify the enrichment of mouse mammary epithelial stem cells, STEMCELL Technologies offers the **EasySep<sup>®</sup> Mouse Mammary Stem Cell Enrichment Kit** (Catalog #19757) for the purification of mouse mammary stem and progenitor cells.

The EasySep<sup>®</sup> Mouse Mammary Stem Cell Enrichment Kit relies on a two-step process, starting with immunomagnetic cell separation reagents targeting CD45, Ter119, and CD31 to deplete over 90% of the hematopoietic and endothelial cells found within the freshly dissociated mammary gland. The resulting epithelial-enriched fraction can be further enriched for progenitor and stem cells by fluorescence activated cell sorting using the fluorochrome-conjugated anti-CD49f and anti-CD24 antibodies also included in the kit. Representative results from the EasySep<sup>®</sup> Mouse Mammary Stem Cell Enrichment Kit are shown in Figure 1. The MRU-rich fraction is found in the subset of cells expressing high levels of the CD49f and intermediate levels of CD24<sup>1,2</sup> (CD49f<sup>++</sup>CD24<sup>+</sup>). The frequency of MRUs in the CD49f<sup>++</sup>CD24<sup>+</sup> fraction ranges between 1/60 to 1/90. Ma-CFCs are found in the subset of cells expressing intermediate levels of CD49f and high levels of CD24 (CD49f<sup>+</sup>CD24<sup>++</sup>).

\* CD49f is also known as  $\alpha 6$  Integrin. CD24 is also known as Heat Stable Antigen.

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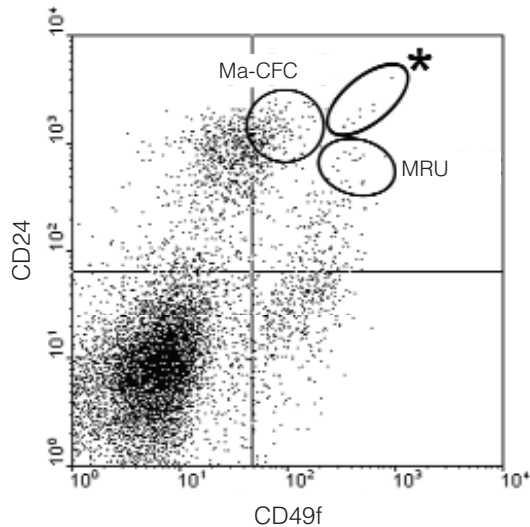
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**FIGURE 1.** Flow cytometry profile of mouse mammary epithelial cells distributed according to their expression of CD49f and CD24. The functionally defined mammary repopulating unit (MRU)-rich fraction is found to be in the subset of cells expressing high levels of the CD49f and intermediate levels of CD24 (CD49f<sup>+</sup>CD24<sup>+</sup>)<sup>1,2</sup> at a frequency ranging between 1/60 to 1/90. The mammary colony-forming cells (Ma-CFCs) express intermediate levels of CD49f and high levels of CD24 (CD49f<sup>+</sup>CD24<sup>++</sup>). In certain strains of mice (such as C57BL/6J) there can be a small number of CD49f<sup>+</sup>CD24<sup>++</sup> cells (indicated by \* in the dot plot). Although mammary stem cells have been detected in this fraction, their frequency is much lower than in the MRU fraction.

The process of “de-bulking” the sample using immunomagnetic cell separation prior to flow cytometry significantly reduces the cell sorting time and permits greater resolution of the various subpopulations (as shown in Figure 1) due to removal of the hematopoietic and endothelial cells (both of which are CD24<sup>+</sup> and CD49f<sup>+</sup>). The result is a viable sample, highly enriched in stem and progenitor cells, that can be subsequently used in functional assays described earlier.

### SUMMARY

Understanding the processes that regulate mammary stem cell development, including the transition from mammary stem cell to mammary progenitor cell and finally differentiated progeny, requires an ability to isolate single cells and assess their ability to proliferate and differentiate. STEMCELL Technologies offers products and protocols for the generation of a single cell suspension from mammary tissue, for the enrichment for specific subsets of mammary epithelial cells and for the evaluation of the functional properties of these discrete subpopulations.

### PROTOCOL FOR MOUSE MAMMARY STEM/PROGENITOR CELL ISOLATION

The following is a recommended protocol for isolation of mammary stem cells and progenitor cells from mouse mammary glands.

1. Harvest inguinal mammary glands from young female mice (8 - 12 weeks old).
2. Dissociate the glands for 6 - 8 hours in Collagenase/Hyaluronidase enzymatic cocktail (Catalog #07912). For complete digestion information, please refer to the Product Information Sheet for Collagenase/Hyaluronidase.
3. Lyse red blood cells using Ammonium Chloride (Catalog #07800/07850).
4. Generate a single cell suspension from the dissociated mammary gland using Trypsin/EDTA (Catalog #07901), Dispase (Catalog #07913) and DNase I (Catalog #07900) enzymes. For complete digestion information, please refer to the Product Information Sheet for Collagenase/Hyaluronidase (Catalog #07912).
5. Filter the suspension through a 40 µm cell strainer (Catalog #27305).
6. Deplete contaminating hematopoietic and endothelial cells using the EasySep Mouse Mammary Stem Cell Enrichment Kit (Catalog #19757). For complete instructions, refer to the Product Information Sheet.
7. Label remaining cells with fluorochrome-conjugated CD24 and CD49f antibodies, also included in the EasySep<sup>®</sup> Mouse Mammary Stem Cell Enrichment Kit (Catalog #19757).
8. Isolate CD49f<sup>+</sup>CD24<sup>+</sup> or CD49f<sup>+</sup>CD24<sup>++</sup> corresponding to stem cell and progenitor enriched fractions, respectively, using a fluorescence activated cell sorter.

In order to functionally analyze enriched cells, one or more of the following procedures can be performed:

- Progenitor cells can be analyzed functionally with the mammary colony-forming cell assay using EpiCult<sup>®</sup>-B (Mouse) (Catalog #05610), FBS (Catalog #06100) and irradiated NIH 3T3 fibroblasts. For complete information, refer to the Product Information Sheet for EpiCult<sup>®</sup>-B (Mouse).
- Ma-CFC-derived colonies can be analyzed using immunocytochemistry with keratin 18 and keratin 19 antibodies for luminal cells and smooth muscle actin and keratin 14 for myoepithelial cells.  
*Note: Tissue culture imposes changes to the cell surface marker expression and reduces the differences between lineages. The lineage specificity is more distinct with in situ staining.*
- The presence of stem cells can be confirmed using the *in vivo* “Fat Pad” assay.<sup>1,2</sup>

### REFERENCES

1. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ. Purification and unique properties of mammary epithelial stem cells. *Nature* 439: 993-997, 2006. Epub 2006 Jan 4.
2. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Aselin-Labat ML, Wu L, Lindemann GJ, Visvader JE: Generation of a functional mammary gland from a single stem cell. *Nature* 439: 84-88, 2006