PRODUCT DESCRIPTION

EpiCult™-B Medium (Mouse) is optimized for the growth of mouse mammary luminal and myoepithelial cells. It is ideal for the culture and evaluation of mouse mammary epithelial progenitors in the mammary colony-forming unit assay when used in conjunction with an irradiated feeder layer such as NIH 3T3 cells. It can also be used for the enzymatic dissociation of mouse mammary tissue when supplemented with Collagenase/Hyaluronidase.

COMPONENTS

05611 EpiCult™-B Basal Medium (Mouse) 450 mL

05612 EpCult™-B Proliferation Supplement (Mouse) 50 mL

Note: Cytokines are not included.

EpiCult™-B Proliferation Supplement (Mouse) is hazardous. Please refer to the Safety Data Sheet (SDS).

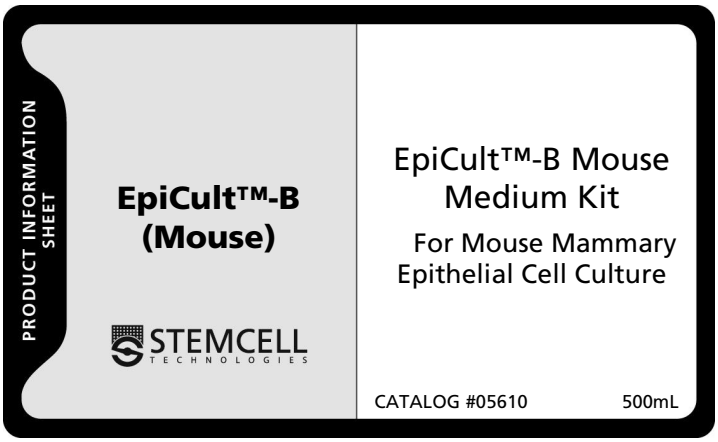
STABILITY AND STORAGE

05611 EpiCult™-B Basal Medium (Mouse)
Store at 2 - 8°C. Stable until expiry date (EXP) on label.

05612 EpiCult™-B Proliferation Supplement (Mouse)
Store at -20°C to -15°C. Stable until expiry date (EXP) on label.

REQUIRED MATERIAL NOT INCLUDED

PRODUCT	CATALOG #
Ammonium Chloride Solution	07800
Collagen Solution	04902
Collagenase/Hyaluronidase	07912
Dispase (5 U/mL)	07913
DNase I Solution (1 mg/mL)	07900
Fetal Bovine Serum (FBS)	06100 or quality cell culture-tested equivalent
L-Glutamine	07100
Hanks' Balanced Salt Solution (HBSS) with 10 mM HEPES, Without Phenol Red	37150
0.2% Heparin Sodium Salt in PBS	07980
Recombinant Human Epidermal Growth Factor (rh EGF)	02633
Recombinant Human Basic Fibroblast Growth Factor (rh bFGF)	02634
Trypsin-EDTA (0.25%)	07901
40 µm Cell Strainer	27305



DIRECTIONS FOR USE

Note: Avoid the use of glass pipettes and tubes when handling mammary epithelial cells. These cells will stick to the glass.

1.0 Preparation of Complete EpiCult™-B Medium (Mouse)

Use sterile techniques to prepare complete EpiCult™-B Medium (Basal Medium + Proliferation Supplement + cytokines + heparin).

1. Thaw EpiCult™-B Proliferation Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   Note: A white precipitate may have formed after storage at -20°C. If the precipitate is present after complete thawing, heat the supplement in a 37°C water bath until the precipitate disappears.

   Once thawed, use immediately or aliquot and store at -20°C for up to 3 months. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

2. Add 50 mL of EpiCult™-B Proliferation Supplement to 450 mL of EpiCult™-B Basal Medium.

   Note: If not used immediately, store complete EpiCult™-B Medium without added cytokines at 2 - 8°C for up to 1 month.

3. Cytokines are not included in the Basal Medium or Proliferation Supplement and should be added immediately before use, along with heparin. STEMCELL Technologies recommends adding:
   - 10 ng/mL recombinant human Epidermal Growth Factor (rh EGF)
   - 10 ng/mL recombinant human Basic Fibroblast Growth Factor (rh bFGF)
   - 4 µg/mL (0.0004%) heparin

   Note: If complete EpiCult™-B Medium is not used immediately, aseptically dispense into working aliquots and store at 2 - 8°C for up to 1 week.

   Complete EpiCult™-B Medium does not contain antibiotics. If desired, they may be added. Following the addition of antibiotics, store complete EpiCult™-B Medium at 2 - 8°C for up to 1 week.

   Avoid repeated exposure of medium to room temperature and light during experiments.

2.0 Dissociation of Mouse Mammary Tissue

1. Prepare dissociation solution by diluting 1 part 10X Collagenase/Hyaluronidase with 9 parts DMEM/F12 (Catalog #36254) or 9 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of the dissociation solution will be required for every 2 mammary glands to be dissociated.

   Note: The choice to dissociate the tissue in DMEM/F12 or in complete EpiCult™-B medium with serum and growth factors will depend on the cells under study. More stem cells are liberated when tissues are dissociated in DMEM/F12, whereas more progenitor cells are liberated when the tissue is dissociated in a serum- and growth factor-containing media.

2. Resect mammary glands and place in the Collagenase/Hyaluronidase-containing dissociation solution (from step 1). It is not necessary to mince the tissue beforehand if the glands are small, but if they are large some mincing of the tissue will help the tissue dissociation. Incubate for 6 - 8 hours at 37°C with occasional pipetting and vortexing.

   Note: Mouse mammary glands can be dissociated for shorter lengths of time (1 - 2 hours), however the mammary stem cell yield is dramatically (~80%) reduced (and in some cases, absent). A short tissue dissociation time results in a high yield of liberated lymphocytes and stromal cells, but the stem cells are apparently lost in the filtration stage (Section 3.0, Step 6), even following trypsin and dispase treatment. Conversely, digestion longer than 8 hours results in over-digestion of the cells and a decrease in stem cell yield (but not total cell yield). Alternatively, Gentle Collagenase/Hyaluronidase (Catalog #07919) may be used for overnight dissociation of mouse mammary tissue.

3. After dissociation, vortex the tissue and triturate with a pipette, then centrifuge the cells at 450 x *g* and discard the supernatant.

4. Resuspend the pellet with a 1:4 mixture of cold Hanks' Balanced Salt Solution (HBSS) with 10 mM HEPES, Without Phenol Red supplemented with 2% FBS and Ammonium Chloride Solution and centrifuge at 450 x *g* for 5 minutes. The resultant pellet contains epithelial cell organoids as well as stromal cells and lymphocytes. To generate a single-cell suspension of mammary epithelial cells, refer to Section 3.0.

3.0 Generation of Single-Cell Suspensions from Dissociated Mouse Mammary Tissue

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA to the partially-dissociated tissue (generated in Section 2.0) and mix by pipetting.

2. Gently pipette up and down with a P1000 micropipettor for 1 - 3 minutes. The sample should become very stringy due to lysis of dead cells and the release of DNA.

3. Add 10 mL of cold HBSS with 10 mM HEPES, Without Phenol Red supplemented with 2% FBS and centrifuge at 450 x *g* for 5 minutes. The HBSS + FBS solution is now referred to as HF.

4. Remove as much of the supernatant as possible.

5. Add 2 mL of pre-warmed 5 U/mL Dispase and 200 µL of 1 mg/mL DNase I Solution. Pipette the sample for 1 minute with a P1000 micropipettor to further dissociate cell clumps. The sample should now be cloudy, but not stringy. If still stringy, add an additional 100 µL of DNase I Solution and pipette as above.

6. Dilute the cell suspension with an additional 10 mL of cold HF and filter the cell suspension through a 40 µm cell strainer into a new 50 mL centrifuge tube. Centrifuge at 450 x *g* for 5 minutes and discard the supernatant.

7. If the cell pellet is heavily contaminated with red blood cells, resuspend the pellet in a 1:4 mixture of cold HF:Ammonium Chloride Solution, centrifuge at 450 x *g* for 5 minutes, and discard the supernatant.

4.0 Culture of Mouse Mammary Epithelial Cells

Mouse mammary epithelial cell cultures should be initiated from single-cell suspensions (refer to Section 3.0), otherwise cells will not adhere well to the tissue culture flask.

1. Seed mouse mammary cells into tissue culture flasks at a density of 2 - 4 x 10^3 cells/cm^2 in complete EpiCult™-B Medium (Mouse) supplemented with cytokines and heparin (as described in Section 1.0), and 5% FBS.

   Note: Failure to include serum during plating of mouse mammary epithelial progenitor cells will result in poor adherence of the cells to the tissue culture plastic.

2. After 24 hours, change the culture medium to serum-free complete EpiCult™-B Medium (Mouse) containing cytokines and heparin.

   Note: Failure to change the medium to serum-free complete EpiCult™-B Medium (Mouse) could result in overgrowth of the culture by contaminating stromal cells.

   Mammary epithelial cells can be sub-cultured 7 - 10 days later by first washing the adherent cells with HBSS with 10 mM HEPES, Without Phenol Red followed by incubation with pre-warmed Trypsin-EDTA (0.25%). Once the cells have detached from the culture vessel, an equal volume of cold HF (refer to Section 3.0, Step 3) should be added and the cell suspension centrifuged at 350 *x g*. Collected cells can then be reseeded into tissue culture flasks as described above in steps 1 and 2.

5.0 Mouse Mammary Colony-Forming Unit Assay

The Mammary Colony-Forming Unit (Ma-CFU) Assay is a commonly used method to quantify the number of progenitors (colony-forming units) in a sample.

1. Mammary cells need to be seeded at clonal density (< 500 cells/cm^2) onto a layer of pre-established irradiated viable feeder cells in complete EpiCult™-B Medium (Mouse) supplemented with cytokines and heparin (as described in Section 1.0), and 5% FBS.

   Note: The use of NIH 3T3 cells irradiated at 5 x 10^3 cGy and seeded at 1 x 10^4 cells/cm^2 is recommended. The NIH 3T3 cells should be derived from sub-confluent cultures.

2. Incubate at 37°C for 6 - 8 days. On the second day, change the culture medium to serum-free complete EpiCult™-B Medium (Mouse) with cytokines and heparin.

   Note: Failure to change the medium to serum-free complete EpiCult™-B Medium (Mouse) could result in overgrowth of the culture by contaminating stromal cells.

3. Fix, stain, and count the CFUs.

6.0 Three Dimensional Extracellular Matrix (ECM) Assay

Mammary epithelial cells can also be cultured in a basement membrane matrix (such as Matrigel®, Corning® Catalog #354277) as described below.

1. Generate a single-cell suspension of mouse mammary cells (refer to Section 3.0).
2. Aliquot a minimum of 5 x 10^4 freshly dissociated mouse cells into a 1.5 mL microcentrifuge tube.
3. Centrifuge at 450 x *g* for 5 minutes and discard supernatant.
4. Add 60 µL of chilled basement membrane matrix (e.g., Matrigel®) to the cell pellet and gently pipette up and down with a 200 µL pipette to resuspend the cells. Avoid creating air bubbles.

   Note: The basement membrane matrix must be kept on ice at all times to prevent gelation.

5. Immediately aliquot 50 µL of the cell + matrix mixture into a tissue culture dish (8-well chamber slide or 24-well plate or 60 mm dish).

   Note: The matrix material will form a 'blob' in the middle of the dish, allowing for retrieval of the matrix for future manipulations, if desired.

6. Place the dish in a humidified 37°C incubator for 10 minutes to promote solidification of the matrix.
7. Gently overlay the solidified gel with pre-warmed complete EpiCult™-B Medium (Mouse) with cytokines and heparin supplemented with 5% FBS such that the gels are well covered (i.e., 400 µL for an 8-well chamber slide; 1 mL for a 24-well plate; 4 mL for a 60 mm dish).

   Note: Although complete EpiCult™-B Medium (Mouse) promotes excellent proliferation of mouse mammary epithelial cells on tissue culture plastic, 5% serum supplementation is required for optimal growth when the cells are cultured in a basement membrane matrix.

8. Spherical acinar and branched ductal structures will develop after about 10 - 14 days *in vitro*. Change medium to fresh complete EpiCult™-B Medium (Mouse) with cytokines and heparin supplemented with 5% FBS when the medium has become acidic (yellow or orange in color).

NOTES

- Enhanced growth of mouse mammary cells can be achieved by pre-coating the tissue culture dish with a thin film of collagen (Catalog #04902).