EpiCult™-B Mouse Medium Kit

For culture and evaluation of mouse mammary epithelial cells

Catalog #05610 500 mL

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Product Description

EpiCult™-B Medium (Mouse) is a serum-free liquid culture medium optimized for the culture of mouse mammary luminal and myoepithelial cells. It is ideal for the culture and evaluation of mouse mammary epithelial progenitor cells in the mammary colony-forming unit (CFU) assay when used in conjunction with an irradiated feeder layer such as NIH 3T3 cells. This medium can also be used for the enzymatic dissociation of mouse mammary tissue when supplemented with collagenase and hyaluronidase. Addition of human recombinant epidermal growth factor (EGF), human recombinant basic fibroblast growth factor (bFGF), and heparin is required for culturing cells.

Product Information

The following components are sold as a complete kit (Catalog #05610) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
EpiCult™-B Basal Medium (Mouse)	05611	450 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
EpiCult™-B Proliferation Supplement (Mouse)*	05612	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Ammonium Chloride Solution	07800
Collagen Solution	04902
Gentle Collagenase/Hyaluronidase	07919
Dispase (5 U/mL)	07913
DNase I Solution (1 mg/mL)	07900
Fetal bovine serum (FBS; quality cell-culture tested)	---
Gentamicin	---
L-Glutamine	07100
HBSS with 10 mM HEPES, Without Phenol Red	37150
Heparin Solution	07980
Human Recombinant bFGF	78003
Human Recombinant EGF	78006
Trypsin-EDTA (0.25%)	07901
40 µm Cell Strainer	27305

Preparation of Complete EpiCult™-B Medium (Mouse)

Use sterile techniques to prepare complete EpiCult™-B Medium (Mouse) (Basal Medium + Proliferation Supplement + EGF + bFGF + heparin). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

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

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.

   NOTE: 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 EpiCult™-B Medium without added cytokines at 2 - 8°C for up to 1 month.
3. Immediately before use, add cytokines and heparin as follows:
   - 10 ng/mL Human Recombinant EGF
   - 10 ng/mL Human Recombinant 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.

Directions for Use

Please read the entire protocol before proceeding.

NOTE: Avoid the use of glass pipettes and tubes when handling mammary epithelial cells, as these cells will stick to glass.

A. DISSOCIATION OF MOUSE MAMMARY TISSUE

1. Thaw Gentle Collagenase/Hyaluronidase at room temperature (15 - 25°C) or overnight at 2 - 8°C.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C until the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Dilute Gentle Collagenase/Hyaluronidase as outlined below for either 2-hour or overnight dissociation.

   NOTE: Approximately 2 - 5 mL of the EpiCult™-B Medium/Collagenase/Hyaluronidase/FBS/gentamicin solution will be required for every 2 mammary glands to be dissociated. Alternatively, mammary glands can be dissociated in DMEM/F-12 with 15 mM HEPES (Catalog #36254) supplemented with 50 µg/mL gentamicin to avoid influences of exogenous growth factors and FBS; however, this may result in lower total viable cell yields.

   2-Hour Dissociation

   Dilute 1 part Gentle Collagenase/Hyaluronidase with 4 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS and 50 µg/mL gentamicin in a 15 mL or 50 mL tube (e.g. Catalog #38009 or 38010). Continue to step 3.

   Overnight Dissociation

   Dilute 1 part Gentle Collagenase/Hyaluronidase with 9 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS and 50 µg/mL gentamicin in a 15 mL or 50 mL tube (e.g. Catalog #38009 or 38010). Continue to step 4.

3. For 2-hour dissociation, resect mammary glands and transfer to a sterile glass Petri dish. Mince with scalpels in a crosswise pattern until glands are rendered to a paste. This step is not necessary for overnight dissociation.

   NOTE: It is essential that the glands are well minced, or total viable epithelial cell yield will be low.
4. Transfer the mammary tissue to the tube containing EpiCult™-B Medium/Collagenase/Hyaluronidase/FBS/gentamicin.

   2-Hour Dissociation: Incubate at 37°C on a rotary/rocking shaker set at 90 rpm for 2 hours. After 1 hour and at the end of incubation, pipette the suspension up and down 20 times using a 1 mL pipettor.

   Overnight Dissociation: Incubate at 37°C for 15 hours (overnight). It is not necessary to use a shaker.
5. Centrifuge the cells at 350 x *g* for 5 minutes and discard the supernatant.

6. Resuspend the cell pellet in a mixture of 1 part cold HBSS with 10 mM HEPES, Without Phenol Red supplemented with 2% FBS and 4 parts Ammonium Chloride Solution and centrifuge at 350 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 B.

B. GENERATION OF A SINGLE-CELL SUSPENSION FROM DISSOCIATED MOUSE MAMMARY TISSUE

1. Add 1 - 5 mL of warm Trypsin-EDTA to the partially-dissociated tissue (generated in section A) and mix by pipetting with a 1 mL pipettor.
2. Gently pipette up and down with a 1 mL 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 will be referred to as HF.
4. Remove as much of the supernatant as possible.
5. Add 2 mL of warm 5 U/mL Dispase and 200 µL of 1 mg/mL DNase I Solution. Pipette the sample for 1 minute with a 1 mL 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.

C. CULTURE OF MOUSE MAMMARY EPITHELIAL CELLS

Mouse mammary epithelial cell cultures should be initiated from single-cell suspensions (refer to Section B), 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) with cytokines and heparin supplemented with 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 as follows: Wash the adherent cells with HBSS with 10 mM HEPES, Without Phenol Red then incubate with warm Trypsin-EDTA (0.25%). Once the cells have detached from the culture vessel, add an equal volume of cold HF (refer to section B, step 3) and centrifuge the cell suspension at 350 x *g*. Collect the cells and reseed into tissue culture flasks as described above in steps 1 and 2.

D. MOUSE MAMMARY COLONY-FORMING UNIT ASSAY

The Mammary Colony-Forming Unit (Ma-CFU) Assay is a commonly used method to quantify the number of progenitor cells (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) with cytokines and heparin supplemented with 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.

Notes and Tips

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