EpiCult™-B Human Medium Kit

For culture and evaluation of human mammary epithelial cells

Catalog #05601 100 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

EpiCult™-B Human Medium Kit is a serum-free culture medium optimized for the culture of human mammary luminal and myoepithelial cells. It is ideal for the growth and evaluation of bipotent, luminal-restricted and myoepithelial-restricted 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. This medium is also ideal for enzymatic dissociation of human mammary tissue when supplemented with Collagenase/Hyaluronidase (Catalog #07912).

This kit contains EpiCult™-B Basal Medium (Human) and EpiCult™-B Proliferation Supplement (Human). Addition of Hydrocortisone Stock Solution (Catalog #07925) is required.

Product Information

The following components are sold as a complete kit (Catalog #05601) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
EpiCult™-B Basal Medium (Human)	05602	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
EpiCult™-B Proliferation Supplement (Human)*	05603	1 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
Collagenase/Hyaluronidase	07912
Dispase (5 U/mL)	07913
DNase I Solution (1 mg/mL)	07900
Fetal Bovine Serum (FBS; quality cell-culture tested)	---
L-Glutamine	07100
HBSS with 10 mM HEPES, Without Phenol Red	37150
Hydrocortisone Stock Solution	07925
Tissue Dissociation Flask	27300
Trypsin-EDTA (0.25%)	07901
40 µm Cell Strainer	27305

Preparation of Complete EpiCult™-B Medium (Human)

Use sterile techniques to prepare complete EpiCult™-B Medium (Human) (Basal Medium + L-Glutamine + Proliferation Supplement + hydrocortisone).

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

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Do not exceed the expiry date as indicated on the label. Do not re-freeze.
2. Add 1 mL of 200 mM L-Glutamine to 100 mL of EpiCult™-B Basal Medium to achieve a final concentration of 2 mM.

   NOTE: L-Glutamine is a labile amino acid with a half-life of approximately 1 month when stored at 2 - 8°C. EpiCult™-B Basal Medium will need to be supplemented with L-Glutamine as described above every 2 months.
3. Add 1 mL of EpiCult™-B Proliferation Supplement to 100 mL of EpiCult™-B Basal Medium + L-Glutamine.

   NOTE: If not used immediately, store EpiCult™-B Medium (Human) without hydrocortisone at 2 - 8°C for up to 2 weeks.
4. Immediately before use, supplement EpiCult™-B Medium (step 3) with 0.5 mL Hydrocortisone Stock Solution to a final concentration of 0.48 µg/mL. This is now complete EpiCult™-B Medium (Human).

Directions for Use

Please read the entire protocol before proceeding.

A. DISSOCIATION OF HUMAN MAMMARY TISSUE

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in complete Epicult™-B Medium (Human) supplemented with 5% fetal bovine serum (FBS).
2. Transfer the tissue to sterile glass Petri dishes, mince with scalpels and then transfer to tissue dissociation flasks.

   NOTE: Glass Petri dishes can be used for this initial dissociation, as the concentration of epithelial cells is very low.
3. Dilute 1 part Collagenase/Hyaluronidase with 9 parts complete Epicult™-B Medium and add to the minced tissue in the dissociation flasks. Ensure that the tissue is well-suspended in the enzyme mixture and the final volume is level with the widest portion of the flask. Cover the opening of the flask with sterile aluminum foil.
4. Gently dissociate the minced tissue on a rotary shaker at 37°C until all large tissue fragments are digested. Typical digestion time is 16 hours (overnight) for normal human mammary tissue. Longer digestion times may be required for tough fibrous tissue, shorter digestion times for softer tissue.

   NOTE: The flasks should be sealed if the rotary shaker is not in a 5% CO_2 incubator.
5. After dissociation, transfer the tissue to 50 mL centrifuge tubes, and centrifuge at 80 x *g* for 30 seconds.
6. Discard the overlying liquefied fat layer. The pellet (“A” pellet) is highly enriched for epithelial organoids. To generate a single-cell suspension from the “A” pellet, refer to section B.
7. Transfer the supernatant to a new 50 mL centrifuge tube and centrifuge at 200 x g for 3 minutes. The pellet (“B” pellet) from this second centrifugation contains variable numbers of epithelial cells, stromal cells and red blood cells. To generate a single-cell suspension from the “B” pellet, refer to section B.
8. The supernatant from the second centrifugation is enriched for human mammary fibroblasts. To collect, transfer the supernatant to a new 50 mL centrifuge tube and centrifuge at 350 x *g* for 5 minutes.
9. The different cell fractions can now be cryopreserved. It is recommended that cells are cryopreserved in complete Epicult™-B Medium supplemented with 50% FBS and 6% dimethyl sulfoxide (DMSO).

B. GENERATION OF SINGLE-CELL SUSPENSIONS FROM DISSOCIATED HUMAN MAMMARY TISSUE

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA (0.25%) to the Collagenase/Hyaluronidase-dissociated mammary cells and resuspend cells.

   NOTE: For human tissue, the best starting materials are “A” pellets (see section A). “B” pellets may also be used; however, the success of the cultures derived from these pellets is more variable due to the variable epithelial content. For mouse cells, use the entire mammary cell pellet.
2. Gently pipette up and down with a 1 mL micropipette 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 350 x *g* for 5 minutes. The HBSS + FBS solution is now referred to as HF.
4. Remove as much of the supernatant as possible. The cells may be a large ‘stringy mass’ floating in the HF.
5. Add 2 mL of pre-warmed 5 U/mL Dispase and 200 µL DNase I Solution. Pipette the sample for 1 minute with a 1 mL micropipette to further dissociate cell clumps. The sample should now be cloudy, but not stringy. If still stringy, add an additional 100 µL 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 HUMAN MAMMARY EPITHELIAL CELLS

Human 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.

NOTE: Enhanced growth of human mammary cells can be achieved by pre-coating the tissue culture dish with a thin film of Collagen Solution (Catalog #04902).

1. Seed human mammary cells into tissue culture flasks at a density of 1 - 5 x 10^4 cells/cm^2 in complete EpiCult™-B Medium supplemented with 5% FBS.

   NOTE: Failure to include serum during plating of mammary cells will result in poor adherence of the cells to the tissue culture plastic.
2. After 24 hours, change the culture medium to complete EpiCult™-B Medium without serum.

   NOTE: Failure to change the medium to serum-free complete EpiCult™-B Medium could result in overgrowth of the culture by contaminating stromal cells.
3. Mammary epithelial cells can be sub-cultured 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, add an equal volume of cold HF (refer to section B, step 3) and centrifuge the cell suspension at 350 x *g*. Collected cells can then be reseeded into tissue culture flasks as described in steps 1 and 2.

Notes and Tips

- Seeding human mammary cells at clonal densities (less than 800 cells/cm^2) should be performed using pre-established irradiated viable feeder layers. The use of NIH 3T3 cells irradiated at 5 x 10^3 cGy and seeded at 10^4 cells/cm^2 is recommended. Pure luminal cell, pure myoepithelial cell, and mixed-lineage colonies will be generated after 7 - 10 days when cultured under these conditions.
- For further information, refer to the Technical Bulletin: Monolayer Culture of Human Mammary Epithelial Cells (Document #29178), available at www.stemcell.com or contact us to request a copy.