

# EpiCult™-B Human Medium Kit

**For culture and evaluation of human mammary epithelial cells in CFU assays**

Catalog #05601

100 mL



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## 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 cells.

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
DMEM/F-12 with 15 mM HEPES	36254
DNase I Solution (1 mg/mL)	07900
EpiCult™-C Human Medium Kit	05630
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.

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

### A. DISSOCIATION OF HUMAN MAMMARY TISSUE

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in DMEM/F-12 with 15 mM HEPES supplemented with 5% fetal bovine serum (FBS).
2. Transfer 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 (10X stock) with 9 parts complete EpiCult™-C Medium (Human) and add to the minced tissue in the dissociation flasks.  
NOTE: Alternatively, mammary tissue can be dissociated in DMEM/F-12 with 15 mM HEPES supplemented with 2% wt/vol fraction V BSA, to avoid influences of exogenous growth factors and FBS; however, this may result in lower total viable cell yields.
4. Ensure that the tissue is well-suspended in the enzyme mixture and the final volume is level with the widest portion of the flask.
5. Cover the opening of the flask with sterile aluminum foil.
6. 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 with Parafilm® if the rotary shaker is not in a 5% CO<sub>2</sub> incubator.
7. Transfer the dissociated tissue to 50 mL conical tubes (e.g. Catalog #38010), and centrifuge at 80 x g for 30 seconds.
8. Discard the overlying liquefied fat layer. The pellet ("A" pellet) is highly enriched for terminal ductal lobular unit (TDLU) epithelial fragments.
9. Transfer the supernatant to a new 50 mL conical 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.
10. The supernatant from the second centrifugation is enriched for human mammary fibroblasts. To collect, transfer the supernatant to a new 50 mL conical tube and centrifuge at 350 x g for 5 minutes.
11. The different cell fractions can now be cryopreserved. It is recommended that cells are cryopreserved in complete EpiCult™-C Medium (Human) supplemented with 50% FBS and 6% dimethyl sulfoxide (DMSO).

### B. GENERATION OF A SINGLE-CELL SUSPENSION 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.
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 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 micropipettor 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 conical 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  $1 - 5 \times 10^4$  cells/cm<sup>2</sup> 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<sup>2</sup>) should be performed using pre-established irradiated viable feeder layers. The use of NIH 3T3 cells irradiated at  $5 \times 10^3$  cGy and seeded at  $10^4$  cells/cm<sup>2</sup> 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](http://www.stemcell.com) or contact us to request a copy.

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