MammoCult™ Human Medium Kit

For culture of human mammospheres and tumorspheres

Catalog #05620 500 mL

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Product Description

MammoCult™ Medium (Human) is a serum-free culture medium optimized for the culture of mammospheres from normal human primary breast tissues and tumorspheres from human breast cancer cell lines.

This kit contains MammoCult™ Basal Medium (Human) and MammoCult™ Proliferation Supplement (Human). For preparation of complete MammoCult™ Medium, Hydrocortisone Stock Solution (Catalog #07925) and Heparin Solution (Catalog #07980) are also required.

Product Information

The following components are sold as a complete kit (Catalog #05620) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MammoCult™ Basal Medium (Human)	05621	450 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
MammoCult™ Proliferation Supplement (Human)*	05622	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
Collagenase/Hyaluronidase	07912
Dispase (5 U/mL)	07913
DMEM/F-12 with 15 mM HEPES	36254
DNase I Solution (1 mg/mL)	07900
EpiCult™-B Human Medium Kit	05601
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
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
Tissue Dissociation Flask	27300
Trypan Blue	07050
Trypsin-EDTA (0.25%)	07901
6-Well Ultra-Low Adherent Plates for Suspension Cultures	27145
37 µm Reversible Strainer, Small	27215
Falcon® Conical Tubes, 50 mL	38010

*L-Glutamine is required for preparation of complete EpiCult™-C Medium and complete EpiCult™-B Medium, which are used in the dissociation of human mammary tissue (section A of Directions for Use) and the CFU Assay (section E of Directions for Use), respectively. For further information, refer to the Product Information Sheet for EpiCult™-B Human Medium Kit (Document #29567) or EpiCult™-C Human Medium Kit (Document #29967).

Preparation of Complete MammoCult™ Medium (Human)

Use sterile techniques when preparing complete MammoCult™ Medium (Basal Medium + Proliferation Supplement + heparin + hydrocortisone). 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 MammoCult™ 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. 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 MammoCult™ Proliferation Supplement to 450 mL of MammoCult™ Basal Medium.

   NOTE: If not used immediately, store MammoCult™ Medium at 2 - 8°C for up to 2 weeks.
3. Immediately before use, supplement MammoCult™ Medium as follows:
   - Add 1 mL of Heparin Solution to reach a final concentration of 4 µg/mL.
   - Add 2.5 mL of Hydrocortisone Stock Solution to reach a final concentration of 0.48 µg/mL.

   Complete MammoCult™ Medium does not contain antibiotics. If desired, they may be added.

   Avoid repeated exposure of complete MammoCult™ 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 HUMAN MAMMARY TISSUE

1. Prepare complete EpiCult™-C Medium (Human).

   NOTE: For instructions on preparing complete EpiCult™-C Medium (Human), refer to the Product Information Sheet (PIS; Document #29967) available at www.stemcell.com or contact us to request a copy.
2. 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).
3. 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.
4. 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% w/v Fraction V BSA, to avoid influences of exogenous growth factors and FBS; however, this may result in lower total viable cell yields.
5. Ensure that the tissue is well suspended in the enzyme mixture and the final volume is level with the widest portion of the flask.
6. Cover the opening of the flask with sterile aluminum foil.
7. 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_2 incubator.
8. Transfer the dissociated tissue to 50 mL conical tubes (e.g. Catalog #38010), and centrifuge at 80 x *g* for 30 seconds.
9. Discard the overlying liquefied fat layer. The pellet (“A” pellet) is highly enriched for terminal ductal lobular unit (TDLU) epithelial fragments.
10. 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.
11. 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.
12. The different cell fractions can now be cryopreserved. It is recommended to cryopreserve cells in complete EpiCult™-C Medium (Human) supplemented with 50% FBS and 6% dimethyl sulfoxide (DMSO).

B. GENERATION OF A SINGLE-CELL SUSPENSION FROM PARTIALLY 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 by pipetting up and down. The best starting material is the "A" pellets. "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 pipettor 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 Dispase (5 U/mL) and 200 µL of DNase I Solution (1 mg/mL). Pipette the sample for 1 minute with a 1 mL pipettor 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 37 µm Reversible Strainer into a new 50 mL conical tube. Centrifuge at 350 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 IN SUSPENSION: MAMMOSPHERE CULTURE

1. Obtain a single-cell suspension from mammary organoids as described in section B.
2. Prepare complete MammoCult™ Medium.
3. Seed single human mammary epithelial cells into Ultra-Low Adherent plates at a density no higher than 4 x 10^3 cells/cm^2 in complete MammoCult™ Medium. In each well of a 6-well plate, the maximum seeding density is 3.5 - 4 x 10^4 cells in 2 mL of complete MammoCult™ Medium.

   NOTE: Ultra-Low Adherent plates must be used for mammosphere cultures. Petri dishes or tissue culture-treated dishes cannot be used as they will allow cells to adhere to the surface of the dish and decrease the rate of sphere formation.
4. Incubate at 37°C and 5% CO_2 for 7 days.
5. Count the number of mammospheres that are larger than 60 µm in diameter. Mammospheres may have a solid or hollow morphology.

   NOTE: Prolonged culture may cause mammospheres with a solid morphology to develop into spheres with a hollow morphology.

D. SUBCULTURE OF MAMMOSPHERES

1. Collect all mammospheres into a 50 mL conical tube and centrifuge at 350 x *g* for 5 minutes.

   NOTE: Mammospheres should be passaged when they are ~60 µm in diameter and before they develop a dark center.
2. Aspirate as much supernatant as possible without disturbing the pellet.

   NOTE: The pellet may be very loose.
3. Set the volume on a 1 mL pipettor with sterile plastic tip to slightly less than the approximate volume of the remaining medium (e.g. if there is 800 µL of remaining medium, set the volume of the pipettor to 700 µL). Pre-wet the tip with medium, then add 0.5 - 1 mL of pre-warmed Trypsin-EDTA (0.25%) to the pellet.
4. Triturate mammospheres by slightly tilting the tip and pressing it against the bottom or side of the tube to generate resistance for breaking up the mammospheres. Rinse the side of the tube during trituration to remove the remaining spheres that are attached to the side of the tube. If some mammospheres remain undissociated after 1.5 minutes (this usually occurs at later passages), trituration can be extended to a maximum of 2 minutes.
5. Add 5 mL of cold HF (refer to section B, step 3) and centrifuge the suspension at 350 x *g* for 5 minutes.
6. Aspirate the supernatant and resuspend the pellet in 1 mL of complete MammoCult™ Medium. Perform a viable cell count using Trypan Blue.

   NOTE: Complete MammoCult™ Medium allows for the generation of primary, secondary, and tertiary mammospheres.

E. COLONY-FORMING UNIT (CFU) ASSAY USING CELLS FROM MAMMOSPHERES

Cells collected from dissociated mammospheres can be seeded into tissue culture-treated dishes and assayed for the presence of colony-forming units (CFUs). CFUs can be detected from primary, secondary, and tertiary mammosphere cultures.

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

1. Prepare complete EpiCult™-B Medium (Human) supplemented with 5% FBS.

   NOTE: For instructions on preparing complete EpiCult™-B Medium (Human), refer to the PIS (Document #29567) available at www.stemcell.com or contact us to request a copy.

2. Add cells from dissociated primary mammospheres onto a pre-established irradiated feeder layer at a density of 2 - 3 x 10^3 cells/cm^2 in complete EpiCult™-B Medium (Human) + 5% FBS.

   NOTE: CFU content can vary between samples. The use of NIH 3T3 cells as feeders (irradiated at 5 x 10^3 cGy and seeded at 1 x 10^4 cells/cm^2) is recommended.

3. Incubate at 37°C and 5% CO_2 for 7 - 10 days. On the second day, change the medium to complete EpiCult™-B Medium (Human) **without** FBS.

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

4. After 7 - 10 days, fix, stain, and count the CFUs.

Related Products

For related products, including dissociation reagents, dyes and stains, and cultureware, visit www.stemcell.com/mammoworkflow or contact us at techsupport@stemcell.com.