

## COMPONENTS:

05621	MammoCult™ Basal Medium (Human)	450 mL
05622	MammoCult™ Proliferation Supplements (Human)	50 mL

Hydrocortisone and Heparin must be added before use (not included; refer to Directions for Use for more information).


This product has been aseptically manufactured using tightly controlled processes and is sterility tested.

It should be kept in mind that this product is a biological reagent and as such cannot be completely characterized or quantified. Some variability is unavoidable.

## RELATED PRODUCTS:

Product	Catalog #
Ammonium Chloride Solution	07800
Collagen Solution (Bovine)	04902
Collagen Solution (Human)	04802
Collagenase/Hyaluronidase Solution (10X)	07912
Dispase (5 mg/mL)	07913
DNase I	07900
EpiCult®-B Medium	05601
Fetal Bovine Serum	06100 or quality cell culture-tested equivalent
L-glutamine	07100
Hanks' Balanced Salt Solution Modified	37150
Heparin	07980
Hydrocortisone	07904
Penicillin and Streptomycin Solution (100X)	07500
Tissue Dissociation Flask	27300
Trypan Blue	07050
Trypsin-EDTA	07901
Ultra-Low Adherent Dishes (5/pack)	27145
40 µm Cell Strainers	27305

**Product Information Sheet**



**MammoCult™ Medium (Human)**  
**For Culture of Mammospheres**

Version 1.0.0

Catalog #05620 500 mL

## STABILITY AND STORAGE:

- 05621 MammoCult™ Basal Medium (Human)  
Product stable at 2 - 8°C until expiry date as indicated on the label (refer to Directions for Use for more details).
- 05622 MammoCult™ Proliferation Supplements (Human)  
Product stable at -20°C until expiry date as indicated on the label. Storage of 10 mL aliquots at -20°C is possible. Storage at 2 - 8°C is not recommended. Repeated freezing and thawing is not recommended.

*A white precipitate may form upon storage at -20°C. The precipitate will disappear after complete thawing at 37°C and mixing the contents well.*

Note: Complete MammoCult™ Medium (Human) without Hydrocortisone or Heparin, prepared as directed in Section 1.0, is stable for 2 weeks at 2 - 8°C. Hydrocortisone needs to be replenished weekly. Avoid repeated exposure of medium to room temperature and light during experiments. If the entire volume is not needed immediately, aliquot into appropriate volumes to be used within 1 week (e.g. 100 mL).

## 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 MammoCult™ Medium (Human)

Prepare Complete MammoCult™ Medium (Human) by adding 50 mL of thawed MammoCult™ Proliferation Supplements (Human; Catalog #05622) to 450 mL of MammoCult™ Basal Medium (Human; Catalog #05621) or 1 mL of MammoCult™ Proliferation Supplements (Human) to 9 mL of MammoCult™ Basal Medium (Human).

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Additional supplementation is required for optimal growth of human mammospheres. Add the following to obtain Complete MammoCult™ Medium (Human):

- 4 µg/mL (0.0004%) Heparin (Catalog #07980)
- 0.48 µg/mL Hydrocortisone (Catalog #07904)

This product does not contain antibiotics. If desired, add 5 mL of Penicillin and Streptomycin Solution (Catalog #07500) to 500 mL of medium to achieve a final concentration of 100 U/mL Penicillin and 100 µg/mL Streptomycin. Following addition of antibiotics, medium should be used within 1 week.

## 2.0 Dissociation of Human Mammary Tissue

1. Transport human mammary tissue (6 - 20 grams per specimen) from the operating room on ice in sterile specimen cups in Complete EpiCult®-B Medium (Catalog #05601) supplemented with 5% fetal bovine serum (FBS; Catalog #06100).

2. Transfer the tissue to sterile glass petri dishes, mince with scalpels and then transfer to tissue dissociation flasks (Catalog #27300).

*Glass petri dishes can be used for this initial dissociation, as the concentration of epithelial cells is very low.*

3. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts Complete EpiCult®-B Medium (Catalog #05601) 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.

*The flasks should be sealed if the rotary shaker is not in a 5% CO<sub>2</sub> incubator.*

5. After dissociation, transfer the dissociated tissue to 50 mL centrifuge tubes, and centrifuge for 30 seconds at 80 x g.

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 3.0.

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 3.0.

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 for later use. It is recommended that cells are cryopreserved in EpiCult®-B (Catalog #05601) supplemented with 50% FBS (Catalog #06100) and 6% Dimethyl Sulfoxide.

## 3.0 Generation of Single Cell Suspensions From Partially Dissociated Human Mammary Tissue

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA (Catalog #07901) to the Collagenase/Hyaluronidase-dissociated mammary cells and resuspend cells by pipetting. 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 P1000 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 Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x g for 5 minutes. The Hanks' + 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 mg/mL Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample for 1 minute with a P1000 to further dissociate cell clumps. The sample should now be cloudy, but not stringy. If still stringy, add more DNase I.

6. Dilute the cell suspension with an additional 10 mL of cold HF and filter the cell suspension through a 40 µm cell strainer (Catalog #27305) into a new 50 mL centrifuge 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 (NH<sub>4</sub>Cl; Catalog #07800), centrifuge at 350 x g for 5 minutes and discard the supernatant.

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#### 4.0 Culture of Human Mammary Epithelial Cells in Suspension: Mammosphere Culture

Obtain a single cell suspension from mammary organoids, as described in Section 3.0, to ensure there is minimal contribution of stromal cells to mammosphere formation.

1. Seed single human mammary epithelial cells at a density no higher than  $4 \times 10^3$  cells/cm<sup>2</sup> into Ultra-Low Adherent dishes (Catalog #27145). The maximum seeding density is  $3.5 - 4 \times 10^4$  cells in 2 mL of Complete MammoCult™ Medium (Human; prepared in Step 1.0) per well of a 6-well dish.

*Ultra-Low Adherent dishes (Catalog #27145) 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.*

2. Incubate cultures in a 5% CO<sub>2</sub> incubator at 37°C for 7 days.
3. Count the number of mammospheres that are larger than 60 µm in size. Mammospheres may have a solid or hollow morphology.

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

#### 5.0 Subculture of Mammospheres

1. Harvest mammospheres after 7 days in culture. Collect the entire culture into a 50 mL conical tube and centrifuge at 350 x g for 5 minutes.

*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. Add 0.5 - 1 mL of pre-warmed Trypsin-EDTA (Catalog #07901). Use a P1000 pipettor with sterile plastic tip and set the volume to slightly less than the approximate volume of the remaining medium (for example: if volume of remaining medium is 800 µL, set the volume of the pipettor to 700 µL to avoid creating bubbles). Pre-wet the tip with medium to reduce cells sticking inside the tip.
4. Triturate mammospheres by slightly tilting the tip and pressing it against the bottom or side of the tube to generate resistance in order to break 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 3.0, 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 (Human) containing Heparin and Hydrocortisone and perform a viable cell count using Trypan Blue (Catalog #07050).

*Complete MammoCult™ Medium (Human) allows for the generation of primary, secondary and tertiary mammospheres.*

#### 6.0 Colony-Forming Cell Assay using Cells from Mammospheres

Cells collected from dissociated mammospheres can be seeded into tissue culture-treated dishes (Catalog #27116/27121) and assayed for the presence of colony-forming cells. Colony-forming cells can be detected from primary, secondary and tertiary mammosphere cultures.

1. Prepare Complete EpiCult®-B Medium (Catalog #05601) supplemented with FBS (Catalog #06100). For information on how to prepare Complete EpiCult®-B Medium please refer to the Product Information Sheet for EpiCult®-B Medium (Catalog #05601), available on our website at [http://www.stemcell.com/technical/product\\_sheets.aspx](http://www.stemcell.com/technical/product_sheets.aspx).
2. Add cells from dissociated primary mammospheres at a concentration of  $2 - 3 \times 10^3$  cells/cm<sup>2</sup> onto a pre-established irradiated feeder layers.

*Note: Colony-forming cell (progenitor) content can vary between samples. The use of NIH 3T3 cells as feeders (irradiated at  $5 \times 10^3$  cGy and seeded at  $1 \times 10^4$  cells/cm<sup>2</sup>) is recommended.*

3. Incubate cultures in a 5% CO<sub>2</sub> incubator at 37°C. Epithelial colonies will be generated after 7 - 10 days when cultured under these conditions.

*Enhanced growth of human mammary cells in the colony-forming cell (CFC) assay can be achieved by pre-coating the tissue culture dish with a thin film of human (Catalog #04802) or bovine (Catalog #04902) collagen.*

Refer to Material Safety Data Sheet for more information.

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**NOTES:**

- **Conversion of g to rpm.** To convert g to rpm, use the following formula:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5}) \times (\text{Radius})}}$$

Where:

- RCF = relative centrifugal force (g)
- RPM = centrifuge speed in revolutions per minute
- Radius = radius of rotor in cm

**BACKGROUND REFERENCES:**

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6. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS: *In vitro* propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* 17: 1253-1270, 2003

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