1. What growth medium and reagents are used to culture human mammary epithelial cell cultures?

Growth Medium: MEGM-mammary epithelial growth medium BulletKit (Lonza #CC-3150, MEBM basal medium plus supplements) plus additional supplements.*

BulletKit Components	Additional Supplements
Bovine Pituitary Extract (~70 µg/ml)	Isoproterenol (10^{-5}M)
Hydrocortisone (0.5 µg/ml)	Transferrin (5 µg/ml)
Insulin (5 µg/ml)	
Epidermal Growth Factor (EGF 5ng/ml)	

*Suppliers: Lonza *(http://www.lonza.com) (800-638-8174).* BulletKit also contains gentamicin, which is not necessary for culture. If MEGM is unavailable, possible alternatives include: MCDB 170 (US Biological #M2162-05) with the above supplements *or* Medium 171 (#M-171-500) plus mammary epithelial Growth Supplement (MEGS, #S-015-5) from Invitrogen.

Isoproterenol: Sigma #I5627 or equivalent. To make 1000X stock, dissolve 37.16 mg (0.0372 g) isoproterenol in 15 ml of 95% ethanol (non-denatured) and filter sterilize using a 0.2 µm-pore nylon syringe filter. Dispense 0.5 - 1.5 ml aliquots into sterile 2 ml cryovials and store at -80°C.

Transferrin: Sigma #T8158 or equivalent. To make 1000X stock, dissolve 50 mg (0.05 g) transferrin in 10 ml of PICOpure water and filter sterilize using a 0.2 µm-pore syringe filter. Dispense 0.5 ml aliquots into sterile 1.5 ml microtubes and store at -80°C.

Versene: Versene 1:5000 is 0.2 g/L EDTA•4Na in PBS (Invitrogen #15040-066 or equivalent).

Trypsin-EDTA: 0.05% Trypsin – 0.53 mM EDTA•4Na in Ca^{2+}- Mg^{2+}- Free Dulbecco's PBS (CMF-PBS) or CMF-Hank's Balanced Salt Solution (CMF-HBSS).

Trypsin Stop: (Optional) 0.5 mg/mL Soybean Trypsin Inhibitor (Invitrogen #17075-029 or equivalent) in Ca^{2+} - Mg^{2+} - Free D-PBS or Ca^{2+} - Mg^{2+} - Free HBSS.

Recommended Substrate: (see #3) Collagen type IV (e.g., Sigma #C7521 or #C0543 or equivalent) or pre-coated flasks (BD Biosciences #354534 or equivalent)

2. How is a human mammary epithelial cell line subcultured?

Volumes are for 25-cm^2 flask or 60-mm (20-cm^2) dish

NOTE: It is important to subculture the cells when they are sub-confluent or just confluent, as they lose viability when kept at confluence.

1. Remove medium by aspiration.
2. Rinse cell monolayer with 3 ml versene. Optional: Incubate for 5 minutes. Remove.
3. Incubate cells with 1.0 ml Trypsin-EDTA at 37°C for 5-10 minutes.
4. After ~90% of the cells come loose (with gentle tapping of the flask), add 5 – 6 ml of either Trypsin-Stop or MEGM.
5. Break up cell clumps by gentle trituration.
6. Transfer cell suspension to 15-ml centrifuge tube.
7. Remove aliquot for cell count.
8. Centrifuge recovered cell suspension at 200 x g for 5 minutes.
9. Aspirate supernatant and re-suspend cell pellet in Growth Medium.
10. Inoculate cells at 6 x 10^3 to 2.0 x 10^4 cells/cm^2 in 5 ml MEGM.
11. Re-feed original flask with 5 ml Growth Medium as a backup culture.

3. Is there a recommended substrate?

Yes, collagen type IV (e.g., Sigma #C7521 or #C0543 or equivalent) or pre-coated flasks (BD Biosciences #354534 or equivalent) will improve attachment and growth. See information on preparation, use and coating below.

Collagen IV Preparation and Use*:* Either Mouse Collagen IV from EHS tumor cells (Sigma #C0543) or Human Collagen (Sigma #C7521, "Type VI", Acid Soluble from Human Placenta [Type IV], powder) can be used.

1. To prepare collagen IV from powder, add sterile, 0.5 M acetic acid to the vial to yield 0.5 mg collagen IV per ml, e.g., 10 ml to a 5 mg vial. If larger quantity vials are used, it may be necessary to transfer the dry material to a sterile beaker large enough to contain the required volume of solvent. The material is kept at 4°C for several hours to overnight with occasional gentle swirling until dissolved. **Do NOT shake vigorously or attempt to pipet the material to dissolve!**
2. If it has not dissolved overnight, it may be stirred with a sterile stir bar on a magnetic stirrer at room temperature for periods up to 1 hour, then re-cooled to 4°C. **Do NOT filter!** Aseptically dispense 1ml aliquots and store at -70°C to -80°C.
3. Each of these 0.5 mg/ml stock vials can be diluted 5-fold with sterile, PICOpure water to prepare working stocks at 100 µg/ml that can be frozen and thawed once more. **Do NOT freeze-thaw repeatedly!**

Coating Tissue Culture Vessels with Collagen IV:

1. To coat culture vessels for use with mammary epithelial cells, thaw the required number of vials of collagen IV slowly at 4°C. Each 100 µg aliquot is sufficient to coat (2) 75 cm^2 or (6) 25 cm^2 flasks, or about three 6-well (35 mm) plates.
2. Coating vessels with collagen IV enhances the plating efficiency of mammary epithelial cells. If coating 25 cm^2 flasks, the aliquot is diluted to 15 ml with 14 ml sterile PICOpure water or PBS and 2.5 ml are dispensed per flask. The flasks must be incubated at 37°C for 30 minutes or kept at room temperature for at least one hour prior to use, and may be left in the hood overnight. Flasks may be coated in advance, and refrigerated immediately upon the addition of the collagen solution. They may be stored at 4°C for up to one (1) month before use.
3. Aspirate the collagen solution from the flasks immediately before use and rinse flasks once with calcium- and magnesium-free PBS.

4. What is the freezing medium used to cryopreserve human mammary epithelial cell lines?

Growth medium + 10% DMSO

5. How should human mammary epithelial cell lines be cryopreserved and stored?

1. Place cells into a single-cell suspension, count and pellet as indicated in the subculture protocol above.
2. Resuspend the cells in freezing medium to a seeding density of 5.0e5 viable cells per ml
3. Aliquot 1 ml into each cryovial or ampule.
4. Cells resuspended in freezing medium should be immediately placed in a controlled rate freeze machine that reduces temperature at a controlled rate of -1°C/min. Alternatively, cryovials can be placed in an ethanol bath at -80°C overnight before being placed in liquid nitrogen vapor.

5. Frozen cell stocks are stored in liquid nitrogen tanks. Glass ampules are submerged in liquid, plastic cryovials are stored in vapor phase.

*Suppliers of reagents are listed for the convenience of culture recipients only. Such lists are not intended to be either selective or exhaustive, and Coriell Institute does not recommend specific products or suppliers. Other media and reagents may be satisfactory, but have not been tested.