FREEZING PROTOCOL FOR HUMAN AIRWAY EPITHELIAL CELLS

MATERIALS

- 2X freeze solution
- Ham's F12 medium
- 1.8 ml cryovial

1. Wash cells with PBS, add trypsin and incubate for 3-5 min at 37°C.
2. Harvest cells and neutralize with STI (equal volume). Spin 600g, 5 min at 4°C. Aspirate sup and resuspend in medium.
3. Count cells with a hemacytometer.
4. Adjust volume to a cell density of 1 - 6 million cells/mL.
5. Place cells, 2X Freeze Solution, and cryovials on ice for approximately 10 min to chill.
6. Do not remove 2X Freeze Solution from ice until ready to add to cells.
7. **Slowly** add an equal volume of cold 2X Freeze Solution to the cell suspension (on ice) with gentle mixing. This helps to avoid osmotic shock to the cells as well as disperses the heat generated by solvation.
8. Pipette 1-1.8 mL per cryovial, the volume depending on how many cells you want each vial to contain.
9. Store vial(s) in Mr. Frosty freezing containers and place in -80°C freezer overnight.
10. Transfer vial(s) from -80°C freezer and place in liquid N_2 (-196°C).

THAWING PROTOCOL- (Note: Warm medium must be added in a step-wise manner so that the DMSO concentration gradient is not so steep that DMSO exits the cells too quickly.)

Materials

- Beaker of 37°C water
- Ham's F12 and plating medium
- 15 mL centrifuge tube

1. Warm Ham's F12 and plating medium to 37°C.
2. Thaw the cryovial in a beaker of 37°C water.
3. As soon as the cell supension has thawed, remove the cryovial and wipe off the outside with alcohol.
4. Transfer cells to a 15 mL centrifuge tube.
5. Dilute the cell suspension by slowly adding an equal volume of warm F12 medium. Wait 1 min.
6. Dilute the volume another 1:2 and wait 1 min.
7. Add more F12 medium to fill the tube.
8. Spin at 600g for 5 minutes, 4°C.
9. **Gently** resuspend cells in the appropriate plating medium and perform count and viability
10. Plate according to seeding schedule.
11. **Note**: Freshly thawed cells from freezer should be plated on collagen coated dishes or membranes, which is not necessary for routine passaging.

2X FREEZE SOLUTION

MATERIALS:

For 100 mL:

- 2 mL 1.5 M Hepes Solution (stored at 4°C).
- 10 mL FBS- Fetal Bovine Serum
- 78 mL Ham's F12 (1X)-- (stored at 4°C).
- 10 mL DMSO- Dimethylsulfoxide—(Sigma- D-2650)

In General: 2% 1.5 M Hepes
10% FBS
78% Ham's F12(1X)
10% DMSO

PROTOCOL:

1. Make solution in a laminar flow hood.
2. Place 2 mL of 1.5 M Hepes, 10 mL of FBS, and 78 mL of F12 (1X) into a 200 mL beaker. Mix up and down with pipet.
3. Add 10 mL of DMSO last and **gradually**, to dissipate heat of solvation which may denature proteins in serum. Add approximately 5 drops at a time and slightly shake between additions to mix.
4. Final volume should be 100 mL.
5. Filter solution in a laminar flow hood, using a 0.2 μm filter.
6. Aliquots and store tubes at -20°C.