CELL PASSAGING – Double Trypsinization Protocol for Human Airway Epithelial Cells

MATERIALS:
0.1% Trypsin with 1mM EDTA in PBS (1X)
Soybean Trypsin Inhibitor 1 mg/mL in F12 (1X)
Sterile PBS(1X)
Sterile media
Sterile centrifuge tube

PROTOCOL:

1. Aspirate media from culture dish or flask.

2. Rinse cells with sterile **PBS(1X)** to remove traces of media and serum which can inhibit enzyme activity.

3. Aspirate **PBS(1X)** rinse.

4. Add just enough **Trypsin solution** to cover the cells. (3mls is ideal for a 100 mm dish)

5. Place dish or flask in incubator at 37°C for 3 to 10 minutes.

6. Initially check on detachment at 3 minutes. If no cells appear detached from the substrate, firmly rap the dish or flask on the bench top. If any of the cells are still attached, return the plate to the incubator and continue trypsinization until removal is complete. This should not exceed 10 minutes.

7. After 10 minutes, pipette the **Trypsin Solution** into a sterile centrifuge tube containing an equal volume of **Soybean Trypsin Inhibitor**.

8. If any cells remain on the dish, Repeat Steps 4 through 7.

9. Spin the tube containing the cell suspension and at 1500 RPM for 5 minutes at 4°C.

10. Aspirate the supernatant and re-suspend the pellet in a known volume of media. Gently pipette repeatedly to disperse the cells.

11. Remove 12 μL cells and add to 12 μL trypan blue in microfuge tube, mix well. Add 12 μL to hemocytometer to count cells. (*see* Cell Counting Protocol)

12. An additional spin and re-suspension can be done to further remove any remaining Trypsin from the cells.