CELL PASSAGING

MATERIALS:

- 0.1% Trypsin with 1mM EDTA in 1X PBS
- Soybean Trypsin Inhibitor 1 mg/mL in 1X F12
- Sterile PBS (1X)
- Ham's F12
- Sterile centrifuge tube

PROTOCOL:

1. Aspirate media from culture dish.

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

3. Aspirate **PBS** rinse.

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

5. Place dish 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 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/Cell Solution** into a sterile centrifuge tube containing an equal volume of **Soybean Trypsin Inhibitor**. Rinse dish with F12 to harvest any loose cells remaining on the dish and combine in tube.

8. If any cells remain attached on the dish, Repeat Steps 4 - 7 (**Double Trpsinization**).

9. Spin the centrifuge tube containing the cell suspension and at 600g for 5 minutes at 4ºC.

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

11. Add 12 µL Trypan blue into 96 well plate and combine 12 µL of cells to it, mix well. Add 12 µL to hemocytometer to count cells. (SEE CELL COUNT PROTOCOL).