CELL PASSAGING WITH ACCUTASE

MATERIALS:

- Accutase® (Innovative Cell Technologies, Cat.#AT104)
- 1X Ham's F-12
- Sterile centrifuge tube(s)
- Trypan Blue

PROTOCOL:

1. Carefully aspirate all of the media from the cell culture dish.

2. Add enough Accutase® to the dish to cover the cells.

3. Place dish in a 37°C incubator for 5 to 10 minutes. Check dish frequently to see if all cells have detached.

4. Once the cells have detached, gently tap the plates on the countertop to dislodge any adhered cells.

5. Collect the Accutase®/cell solution into sterile 50 mL conical(s).

6. Rinse the dishes with 1X Ham's F-12 and add wash into the conical.

7. Centrifuge tube containing the cell suspension 600g for 5 minutes at 4^{o}C.

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

9. 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 Counting Protocol).

Note: This solution does not require a neutralization step. Cells should start attaching soon after seeding.