**Preparation**

1. Work in the tissue culture room. Turn the UV light on for 30 minutes before and after working in the biocabinet. Spray each item entering the biocabinet with 70% ethanol including gloves
2. Aliquot enough media and place in a 37C water bath for at least an hour.
3. Label new tissue culture flasks
4. Place 0.05% Trypsin-EDTA in a 37C water bath for 30 minutes before starting

**Cell Dissociation Using Trypsin**

1. Examine the cells under a microscope to ensure the cells are healthy, not overgrown, and free of contamination
2. Remove and discard the culture media from flask
3. Gently rinse the cells with PBS (w/ out Ca +2 and Mg +2 ions) and discard the wash buffer
4. Add enough pre warmed 0.05% Trypsin-EDTA to coat the entire flask. Place the flask in a 37C incubator. Check the flask underneath a microscope every minute until >90% of the cells are detached. Do not exceed exposure to Trypsin for period longer than 10 minutes
5. Add 2 volumes of pre warmed media to inactivate the trypsin. Gently disperse the medium by pipetting over the cell layer several times
6. Transfer the cell suspension to a conical tube and centrifuge at 150\*g for 5 minutes. Remove the supernatant and resuspend in 10 mL of pre warmed media
7. Dilute the cell suspension to an appropriate seeding density (1:10 generally works well) and add to a new tissue culture flask. Incubate at 37C