CELL CULTURE-RELATED PROTOCOLS

C1: Protocol for cell line establishment from embryonic tissues

1) Sacrifice mouse using approved procedures
2) Immediately swab the animal with 70% alcohol for sterility.
3) With sterile forceps and scissors, make a horizontal incision across the abdomen and under the forelegs.
4) With sterile scissors and forceps remove the embryos.
5) Place embryos in Petri dish containing sterile 1X PBS
6) Rinse tissues until the solution is clear.
7) Dissect out the appropriate tissues and place them in separate wells containing sterile PBS.
8) Under sterile conditions, transfer tissue to separate 25ml sterile flasks containing 5-10ml 0.05% trypsin in HBSS.
9) Stir gently in an incubator at 37 degrees for 15 min.
10) Using sterile 5ml pipette, pipette up and down the solution to further break-down the remaining tissue.
11) Remove the solution containing suspended cells to a sterile 50ml conical plastic centrifuge tube containing 1ml of calf serum per 10ml of suspension to inactivate the trypsin.
12) Centrifuge the pooled cells suspension at 1200 rpm for 5 min. and discard the supernatant
13) Resuspend the cells pellets in fresh growth media and centrifuge again as in step 1
14) Wash the cells severals times with growth media until the supernatant is clear.
15) Resuspend the final pellets in 5-10ml DMEM containing 10% FCS + antibiotics + high glucose + L-glutamine. Count cells in hemacytometer
16) Plate the cells at 10,000,000 cells per 75 cm2 flask containing culture media and incubate at 37 degrees in a humidified CO_2 incubator
17) Change media after 24 hrs and the every other day until cells reach confluence.

C2: Harvesting Cells with Trypsin/EDTA

1) Aspirate media and rinse cells with 5ml sterile 1X PBS.

2) Add 4 ml of Tryp/EDTA(0.05% tryp/EDTA) and incubate at 37 degrees for 3-5 min.

3) Add equal amount of growth media (RPMI or DMEM) containing 10% FBS-Amp/Strep

4) Transfer cells to a sterile tube and centrifuge at 1200 RPM for 5 min. at 4 deg.

5) Aspirate media off and add 5-10 ml of growth media (RPMI or DMEM) containing 10%FBS-amp/strep.

6) Split appropriately into 75cm2 flasks containing 10ml growth media (RPMI or DMEM) containing 10%FBS-amp/strep

7) Place cells in the CO2 incubator.