CMBL – CRJ/JJR
6/10/08 perliminary

Protocol: Thawing Cells

Materials

- Frozen cells
- Complete medium* (cell specific, see below)
- 37^{o} C Water bath
- Tissue culture dishes
- Serological Pipets
- Pipet aid
- Sterile Transfer pipets (e.g. Fisher Sci cat# 13-711-20)
- Ice bucket
- Cryo vial tube rack
- Biohazard bag
- 70% ethanol
- Kimwipes
- Markers
- Nitrile/latex gloves
- Insulated gloves
- Eye/face protector

Procedure

1. Heat complete medium to 37^{o}C in a water bath; partially fill ice bucket with ice
2. Using the liquid nitrogen (LN) log book, identify the location of the desired cells in the nitrogen dewar
3. Prepare the hood:
   - place nitrile or latex gloves on hands
   - spray and wipe the hood surface with 70% ethanol
   - place the following materials into the hood: pre-warmed complete medium, cryo vial tube rack, tissue culture (TC) dishes (1 to 2 dishes per vial to be thawed), sterile transfer pipets, pipet aid, pipets
   - tape a biohazard bag to the front of the hood
4. Place 9 mL of complete medium into each dish and place dishes in the incubator
5. Check the LN log book again to note the rack, box, and vial number of desired cells (note cap colors if indicated)
6. With face protection and gloves on:
   - turn off the LN level meter (if present) and removed the dewar lid
   - slowly pull the rack out of dewar (allowing LN trapped in the boxes to spill back into the dewar)
   - place the rack on a level surface and promptly remove the desired cell vial
   - carefully place rack back in LN dewar and turn on LN level meter
7. Thaw cryo vial by agitating in a 37^{o}C water bath (~1 min.)
8. Once thawed, place vial on ice
9. Remove warm dishes from incubator and place in hood
10. Thoroughly spray cryo vial with 70% ethanol and place in hood
11. Using a sterile transfer pipet, gently mix and place the contents of each cryo vial into 1 or 2 TC dishes
12. Label, gently swirl (to distribute cells), and place dishes back in the incubator

24 hrs. later

13. Observe cells under the microscope (check for contamination, note approx. % of adherent cells)
14. Wash cells 2X with PBS, and place fresh medium on cells
    - use pre-warmed PBS and medium; prepare hood as above

**Complete Media (cell line specific)
MC3T3-E1: αMEM (Invitrogen cat# 12571) + 10 % Fetal Bovine Serum + 1% Pen/Strep (Invitrogen cat# 15140)
FAK - /- ; +/+: αMEM w/out nucleosides (Invitrogen cat# 12561) + 15 % Fetal Bovine Serum + 1% Pen/Strep
NIH 3T3: DMEM (Invitrogen cat# 11995) + 10% Calf Serum + 1% P.S.
See ATCC website for other cell lines (www.atcc.org)