Freezing Cells

FREEZING PROTOCOL:

1. Trypsinize, wash, and resuspend cells in media (see Cell Passaging Protocol if more detail needed)
2. Count cells
3. Adjust volume to a cell density of 1 to 6 million cells/mL
4. Label vials
5. Place cells and 2X Freeze Solution on ice for approximately 10 minutes.
6. Add ice to a small bucket. Place cell vial(s) in Cryotube rack on ice to keep cool. Do not remove 2X Freeze Solution from ice until ready to add to cells.
7. **Slowly** add an equal volume of cold 2X Freeze Solution to the cell suspension (on ice) with gentle mixing. This helps to avoid osmotic shock to the cells as well as disperses the heat generated by solvation.
8. Pipette calculated mL per cryovial, the volume depending on how many cells you want each vial to contain.
9. Put vial(s) in a sealed Styrofoam box or Nunc freezer containers (helps to avoid a too rapid cooling) and place in -80°C freezer overnight.
10. Transfer vial(s) from -80°C freezer and place in liquid N₂ (-196°C ).

**MATERIALS**

- 2 X Freeze Solution
- Media, such as Hams F12
- 1.8 ml Cryovial
- Cryotube rack
Thawing Cells

THAWING PROTOCOL - (Note: Warm media must be added in a step-wise manner so that the DMSO concentration gradient is not so steep that DMSO exits the cells too quickly.)

1. Warm Ham’s F12 and Plating media to 37°C.
   (aliquot and then put in waterbath)
2. Thaw the cryovial in a beaker of 37°C water.
   (use waterbath water)
3. As soon as cell suspension has thawed, remove the cryovial and wipe off the outside with alcohol.
4. Transfer cells to a 15 mL centrifuge tube.
5. Dilute the cell suspension by slowly adding an equal volume of F12 media. Wait 1 min.
6. Dilute the volume another 1:2 and wait 1 min.
7. Add more F12 media to fill the tube.
8. Spin tube at 1500 RPM (600g) for 5 minutes.
9. Gently resuspend cells in the appropriate plating media and perform count and viability
10. Plate according to seeding schedule.

Note: Freshly thawed cells from freezer should be plated on collagen counted dishes or membranes, which is not necessary for routine passaging.

Materials
- Beaker of 37°C water
- Hams F12 and Plating media
- 15 mL centrifuge tube
2X FREEZE SOLUTION

MATERIALS:

For 100 mL:
- 2 mL 1.5 M Hepes Solution (stored at 4°C).
- 10 mL FBS- Fetal Bovine Serum
- 78 mL F12 (1X)-- (stored at 4°C).
- 10 mL DMSO- Dimethylsulfoxide—(Sigma- D-2650), kept at rm temp

In General: 2% 1.5 M Hepes
10% FBS
78% F12(1X)
10% DMSO

PROTOCOL:

1. Make solution in a laminar flow hood.
2. Place 2 mL of 1.5 M Hepes, 10 mL of FBS, and 78 mL of F12 (1X) into a 200 mL beaker. Mix up and down with pipet.
3. Add 10 mL of DMSO last and gradually, to dissipate heat of solvation which may denature proteins in serum. Add approximately 5 drops at a time and slightly shake between additions to mix.
4. Final volume should be 100 mL.
5. Filter solution in a laminar flow hood, using a 0.2 μm filter into a sterile 100 mL bottle.
6. Place 10 mL aliquots of solution into 10 sterile 15 mL centrifuge tubes. Pipet solution very slowly.
7. Store tubes at -20°C