FREEZE/THAW PROTOCOL

Materials:

- 2X Freeze Solution
- Ham's F12
- 1.8 Nunc Cryovials/caps
- Nunc Cryovial rack (starfeet)
- CoolCell freezing container or substitute
- Ice buckett

FREEZING PROTOCOL:

1. Follow Cell Passaging Protocol: wash cells with PBS/add trypsin* and incubate until cells detach. Harvest cell solution and neutralize in equal volume of STI, rinse dish and add to cell solution and centrifuge. *OPTION: Accutase.
2. Resuspend cells in F12 or other medium and count cells.
3. Adjust volume to a cell density of 1 to 6 million cells/mL.
4. Label cyrovials with Code/passage/# cells/date/initials and label caps with numbers of cells.
5. Cells should always be on ice. Keep thawed 2X freeze solution on ice until ready to use.
6. Fill ice bucket and place cyrovial rack on ice to chill (10 min). 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 CoolCell or Nunc freezer containers and place in -80°C freezer overnight.
10. Transfer vial(s) from -80°C freezer and place in liquid N_2 (-196°C).

FREEZE/THAW PROTOCOL

Materials:

- Beaker of 37ºC water
- Warm Ham's F12 or plating media
- 15 mL centrifuge tube

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 37ºC.
2. Thaw the cryovial in a beaker of 37ºC water.
3. As soon as cell supension has thawed, remove the cryovial and wipe off the outside with 70% ETOH.
4. Transfer cells to a 15 mL centrifuge tube.
5. Slowly dilute the cell suspension by adding an equal volume of warm 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 600g for 5 minutes, 4 ºC, and discard the supernatant.
9. **Gently** resuspend cells in the appropriate warm plating media and perform cell count and viability.
10. Plate according to seeding schedule.

Note: Freshly thawed cells should be plated on collagen coated dishes or membranes. This step is not necessary for routine cell passaging on dishes but all membranes should be collagen coated.

FREEZE/THAW PROTOCOL

2X FREEZE SOLUTION

MATERIALS:

For **100 mL**:

- 2 mL 1.5 M Hepes Solution
- 10 mL FBS- Fetal Bovine Serum
- 78 mL F12 (1X)
- 10 mL DMSO- Dimethylsulfoxide—(*Sigma- D-2650*)
- 0.2µm fitler unit

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

PROTOCOL:

1. Make solution in a biological cabinet.
2. Place 2 mL of 1.5 M Hepes, 10 mL of FBS, and 78 mL of F12 (1X) into a 250 mL beaker. Stir.
3. Place beaker on ice and add 10 mL of DMSO last and **gradually**, to dissipate heat of solvation which may denature proteins in serum. Add approximately 5-10 drops at a time and slightly shake between additions to mix.
4. Final volume should be 100 mL.
5. Filter solution in a biological cabinet, using a 0.2 µm filter unit.
6. Aliquot 10 mL of 2X freeze solution into 15 mL conceals tubes.
7. Store tubes at -20°C.