Mack Cell Thawing

General Notes

- Mack cells can be thawed without laminin, but the survival rate is lower without laminin

Materials

- Mack cell cryovial
- 15 mL conical tube
- Mack GM
- iMatrix recombinant laminin-511-E8
    - Iwai North America #N892021
    - 0.5 mg/mL
- T25 or T75 cell culture flask, vented

Method

1. Warm 9 ml of growth media in a 15 ml conical tube to room temperature.
2. Get vial of cells and place in room temperature water bath for ~2 minutes.
    a. Vial is ready for the next step when the ice block starts to become liquid, or when there's liquid and a large piece of ice floating in it.
    b. Once the ice starts melting/thawing, the whole solution will very quickly follow so don't wait in the water bath until it's almost completely thawed.
2. Spray down the vial thoroughly with ethanol before placing in the biohood.
3. Slowly dispense 1 ml of warmed media from the 15 mL conical tube into the bottom of the cryovial, then pipette up liquid from the bottom of the vial and dispense it near the top of the liquid.
    a. This is so that the cells don't immediately go from high solute DMSO media into physiological solute media
    b. i.e to slowly reduce the osmolarity of the freezing media and improve cell viability during thawing
    c. BE CAREFUL DON'T LET VIAL OVERFLOW WHEN ADDING LIQUID/PIPETTING
4. Transfer the cell suspension to the rest of the growth media in the 15 ml conical, mix well to make a homogenous solution.
    a. You can also rinse the empty vial with more of the growth media to ensure that all cells are acquired.
5. Spin down your 15 mL conical tube with cells at 300g for 5 minutes.

Now, you can choose whether to seed with laminin or not.

6. Carefully aspirate Mack GM using an aspirating pipette connected to vacuum in the biosafety cabinet.
7. [With laminin] Resuspend cell pellet in 12 mL Mack GM. Add 37.5 µL of 0.5 mg/mL laminin-511. Seed cell suspension in T75.

8. [Without laminin] Resuspend cell pellet in 4 mL Mack GM. Seed cell suspension in T25.
9. To spread cells somewhat evenly in the flask, swirl flask in full figure-8 motion (~10 times).
10. Place flask in 27°C incubator, without CO_2.
11. Let cells adhere for at least 24 hours, undisturbed.
12. Begin checking flasks for confluence and passage when cells reach 70-80% confluence.