Thawing a Cell Line

Introduction

Thawing a cell line from -80C.

Materials

- DMEM (warm at 37 C before take cells from liquid nitrogen)
- Facon tubes

Procedure

Thawing a Cell Line

1. Fill conical with appropriate media for adding cells after thaw (9 mL of media - 1 mL will be the cells volume). Place conical in **37 degrees C** water bath for **10 minutes.**

2. Pull out log for liquid nitrogen tank and identify rack, box, and row of intended cell line.

3. Using the special gloves in the same drawer as the log, open up liquid nitrogen tank and let stand for a moment. Identify rack and SLOWLY tilt and remove from tank. Place rack on bench and retrieve box. Acquire intended vial and replace box and rack SLOWLY into the tank. *Liquid nitrogen will flow out of the tank if replaced too quickly.*

4. Grab the cell line and thaw it in the watter bath. Careful to not touch the ring and cap in the water. It will take around 2 minutes to thaw, you can do slow circle moviments to help the thawing process.

5. Spray the vial with ethanol 70% before opening. After that, add the cell volume to the falcon tube for centrifugation for removal of the cell freezing media.

6. Centrifuge it at 125 x g for 5 minutes, remove suppernat and leave the pellet untoached. Add 5 mL of DMEM and mix gently. Transfer that volume to a T-25 flask.

7. Place in the 37 degrees C incubator until the next day.

8. Observe the grow and decide on expand the cells to a T-75 flask and so on.