

## Freezing Cells

**CHECK THE LIQUID NITROGEN LEVEL** - this process uses A LOT of nitrogen

### **Making cell freeze media:**

1. 50ml of the appropriate media (individual for each cell type)
2. This media needs **20%** serum. Note: The stock media used for tissue culture usually already has 10% serum, so the freeze media really only need 5ml (or 10% serum) added.
3. Add 5ml (or 10%) **tissue culture grade DMSO** (usually in glass ampules. You should also note the supply of this leftover and replace it as soon as possible.)
4. Put this in the refrigerator or in the ice.

### **Labeling the vials:**

1. Vials need to be labeled the following way:
  - cell name
  - passage number
  - date
  - concentration
  - ATCC code (if there is one)
  - the initials of the person doing the freezing
  - Add this information to the LN Tank Inventory

### **Preparation for freezing:**

1. Put ice in the Corning vial holding device and place the labeled vials into the slots.
2. Place tray on the ice and loosen the caps of the vials
3. You should also have a bucket of ice to put the cold freeze media in
4. Have the appropriate stock media, PBS, and Trypsin/EDT A also in the hood

### **Once you are prepared to start:**

1. Check confluence of cells
2. Aspirate off media
3. Wash with PBS about 10 ml
4. Aspirate off media
5. Add 2 ml of Trypsin/EDTA
6. Incubate flask for 5 min.
7. Count cells.
8. Calculate how many vials needed for each to have  $10^6$  cells.
9. Add that amount to a 15 ml sterile centrifuge tube.
10. Spin for 7 min at setting #4
11. Aspirate off the media
12. Add the appropriate amount of cold freeze media (**1 ml** for each vial)
13. Resuspend the pellet -GENTLY! TEMPERATURE AND TIME ARE CRITICAL!
14. Aliquot 1ml of the cell suspension into each of the LABELLED, COLD 1.5ml cryovials
- 15 Close the caps, move vials to the cold cell freezing apparatus and crank up to the top notch.
- 16 Crank down two notches (~ 6 full turns) every 20-30 min until you get to the bottom.
- 17 Once vials are at the bottom allow cells to freeze for 1 additional hour.

18 Remove vials from the freezing apparatus and place in cryovial holders and put them into the liquid nitrogen.

**Documentation:**

**PLEASE** make sure you document what you freeze in the frozen cell inventory computer file.

This is in Google Docs:

[https://docs.google.com/spreadsheets/d/1ge9ayyZVuSRdl35X8luKF4diiJ-5q\\_ge/edit?gid=888735165#gid=888735165](https://docs.google.com/spreadsheets/d/1ge9ayyZVuSRdl35X8luKF4diiJ-5q_ge/edit?gid=888735165#gid=888735165)