Protocol 004 -- Thawing Chordoma Cell Lines

Introduction

This is a general, go-to, protocol outlining thawing procedures for chordoma cell lines. Any steps deviating from this protocol will be documented in the ELN entry.

Materials

- Mr. Frosty
- 70% ethanol
- 15mL conical
- Appropriate media
- RT Collagen I coated T75 Flask(**when applicable**)

Procedure

Thawing Procedure

1. Remove cryovials from LiN2 and place in -80°C Mr. Frosty to chill down slowly while transferring to biosafety cabinet.
2. Remove cryovials from Mr. Frosty and spray with 70% ethanol, place in hood, and allow ethanol to evaporate. Do not wipe with Kim Wipe.
3. Add **8 mL of RT media** to 15mL conical.
4. Using a 2mL aspirating pipet, aspirate liquid from thread region of the cryovial.
5. When pellet starts to melt, add 1mL of (STEP 3) media to the cryovial, immediately pull up and add back to (STEP 3) 15mL conical. Repeat until frozen pellet is liquid and all liquid is removed from cryovial. This will give the cells fresh growth factors and dilute the DMSO in one step.
6. Spin cells at **250x g for 5 min**
7. Aspirate media. Take care in not aspirating cell pellet as some chordoma cell lines seems to dissociate from cell pellet quickly.
8. Add **12 mL of media** to resuspend cell pellet and transfer to T75 flask(RT Collagen I coated if necessary). Cell seeding density will be around 40% confluency.
9. Incubate at **37°C** and **5% CO_2**.
10. Change media after 3 days to allow cells to adhere.