Protocol 013 - Freezing Chordoma Cell Lines (general)

Introduction

This is a general protocol for freezing chordoma cells lines

Materials

- Cell culture media
- FBS
- DMSO (filtered sterile)
- 2mL Cryovial
- 15mL/50mL conical
- 0.2um nitrocellulose filter
- Appropriate size syringe for filter
- Mr. Frosty with fresh isopropanol

Procedure

Freezing Procedure

1. CRITICAL **Cells should be frozen when they are ~80% - 90% confluent in a vessel that was dedicated for freezing. The cells should also be tested for mycoplasma to circumvent freezing contaminated lines**

2. Make 20% FBS and 10% DMSO freezing media

    a. One T75 flask = three cryovials = 3 mL of freezing media (1 mL / cryovial)

    b. Since we are filtering and volume will be lost, we will need to make a couple mL's extra

    c. So, one 5 mL of freezing media = one T75 flask = 4 mL of 10% FBS media + 500 uL of FBS + 500 uL of DMSO (sterile filtered)

    CRITICAL d. Filter the freezing media sterile with a 0.2um nitrocellulose filter ***use nitrocellulose filter for DMSO compatibility***

3. Label the cryovials with:

    cell line name

    passage number

    % confluence when thawed in a T75 flask (so 30% if three cryovials were made from a single 90% confluent T75 flask)

    date

4. Aspirate media from 80% - 90% confluent flask

5. Wash cells with appropriate amount of PBS (8 mL / 75 cm^2) one time and aspirate PBS fully from flask
6. Add appropriate amount of trypsin (3 mL / 75 cm^2) and place flask in the incubator at 37°C and 5% CO_2 until cells have fully detached
7. After cells have fully detached, resuspend them in an appropriate volume of media (8 mL / 75 cm^2)
8. Spin the cells at 250 rcf for 2.5 min
9. Carefully aspirate the supernatant without disrupting the cell pellet
10. Resuspend the cell pellet in 3 mL of freezing media and aliquot 1 mL of the suspension to each cryovial, so three cryovials in total
11. Place the cryovials in the Mr. Frosty and store overnight in the -80°C
12. The next day remove the cryovials with frozen cell suspension to the LiN2 for long term storage