Wallert and Provost Lab

Excel with hard work and inquiry

Cell Freezing Protocol

Introduction: Cells must be treated very carefully when freezing. Ensure that the cells are healthy by inspection in the phase contrast microscope.

Culture cells in T-75 flasks and freeze when they are no more than 80% confluent or less than 50%

We typically freeze several flasks at a time. This will all be combined after spinning and frozen down as one lot.

Protocol:

- Trypsinize cells in 2 ml trypsin per dish as normal
- Add 5 ml of complete media per dish and triterate.
- Add all cells 15 ml sterile falcon tubes
- Centrifuge at 500 rpm or low setting for 5 min. If there isn't an obvious pellet centrifuge again. ***Avoid hard and extended centrifugations***. The cells are fragile at this time.
- Remove media and add 6 ml of freezing media (per T-75) to the cell pellet (see below for recipe). *You may need to use a different volume of freezing media for different cells.*
- Carefully resuspend by pipet and place one ml each in a well labeled cryo vial.
- Place the vials into the Nalgene cryo freezing container and put the container in the -80°C overnight.
- After 24 hrs, transfer the cells to the LN2 container.

Freezing Media (9ml complete media 1 ml DMSO – dimethylsulfoxide)