Protocol: Freezing Cells

Freezing Cell Lines

Lucy Cherbas

We strongly recommend that you prepare a few ampoules of frozen cells as soon as your cells are growing well. Stored in liquid nitrogen, the cells are stable indefinitely. We use the technique described below.

Materials:

- Freezing medium: M3+BPYE+20% FCS + 10% DMSO. Sterilize by filtration; store at 4°C.
- Sterile cryovials: A variety of brands are available from standard laboratory supply companies.
- A wide-mouth Dewar flask with a tight-fitting stopper.
- A -70°C or -80°C freezer.
- A liquid nitrogen storage tank.

Procedure

- Start with a healthy culture at approximately $5x10^6$ cell/mL (mid-exponential growth). Collect the cells by centrifugation; discard the medium (supernatant).
- Resuspend the cells in freezing medium, 0.25 x the original volume, to give a final concentration of approximately $2x10^7$ cells/mL. To resuspend cells, pipet the pellet up and down with a Pasteur pipet or a serological pipet.
- Dispense the cell suspension into cryovials, 0.5 mL per vial.
- Please the tightly sealed vials of cell suspension into a room temperature Dewar flask. Cap the flask; tape the cap to make sure that it does not fall off.
- Place the Dewar flask into a -80°C freezer for 2-3 days to allow the ampoules to slowly cool to the temperature of the freezer.
- Transfer the frozen ampoules to liquid nitrogen. After the ampoules have had at least an hour to equilibrate to liquid nitrogen temperature, thaw one ampoule to check for sterility and viability of the cells.