PR18: Cryopreservation and thawing of cells

Cryopreservation of Cells

All reagents should be at room temperature to maintain the metabolic activity and membrane fluidity of the cells.

Materials and Equipment

- Cells from human blood sample
- Dimethyl Sulfoxide (DMSO) suitable for cell culture
- Serum
- Cryogenic vials, 1.8 ml (sterile)
- 50 ml conical tubes (sterile)
- Serological pipettes of appropriate volumes (sterile)
- Hemocytometer and microscope for cell counting
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers
- Nalgene 'Mr Frosty' freezing container, filled with Propan-2-ol (isopropanol)
- -80^{o}C Freezer
- Liquid nitrogen storage tank, with holders for 1.8 ml cryogenic vials

Preparation

Prepare a Freezing Solution of 10% DMSO in serum.

Label an appropriate number of 1.8 ml cryotubes based on the anticipated cell count (1-2 million cells per ml blood drawn). Each tube should contain up to 20 million cells.

Procedure

1. **Centrifuge cells at 400 x *g* for 10 minutes.**
2. **Aspirate supernatant from cell pellet and resuspend the cells in residual liquid by tapping the tube until no clumps are visible. Add Freezing Solution slowly (i.e. dropwise) while swirling cells gently: final cell concentration should be approximately 1-2 x 10^{7} cells/ml**
3. **Mix the cells gently by tapping the tube without using a pipette**; avoid foam formation.
4. **Aliquot 1ml PBMC suspension into each pre-labeled cryovial.** Pipette gently and slowly to minimize shear forces.
5. **Place cryovials into a room temperature Nalgene freezing container filled with propan-2-ol.**
6. **Transfer the freezing container to a -80^{o}C freezer for a minimum of 12 hours.**
7. **Transfer the cryovials into liquid nitrogen for indefinite storage.**

Thawing cryopreserved cells

Materials and Equipment

- Cryopreserved cell sample
- 50 ml conical tubes (sterile)
- Serological pipettes of appropriate volumes (sterile)
- Cell culture medium (e.g. RPMI 1640 containing 10% serum, 2mM L-Glutamine, 100U/ml Penicillin and 0.1 mg/ml Streptomycin) warned to 37^{o}C
- Benzonase Nuclease
- 37°C water bath
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure

1. **Prepare cell culture media, containing 10U/ml Benzonase Nuclease, per cell sample to be thawed.**
2. **Warm the cryovial rapidly (e.g. in a waterbath) until only a small amount of ice remains in the cryovial. Remove from the bath and slick the tube gently to completely thaw the sample.**
3. **Transfer the cells from the cryovial into a 50 ml conical tube.**
4. **Dilute the cells in 10x volume of cell culture media + Benzonase.**
5. **Centrifuge cells at 330 × *g* for 10 minutes**.
6. **Aspirate supernatant and gently resuspend the cell pellet in residual liquid**
7. **Repeat wash steps 4 and5**
8. **Resuspend cells in cell culture medium to a final known volume.**
9. **Count live cells using a hemocytometer and light microscope if desired.**
10. **Proceed to allocation of cells for cell culture, staining for flow cytometry or bead sorting.**