

Peripheral Blood Immunophenotyping: Freezing of isolated PBMCs

Protocol for freezing and storage of PBMCs isolated from whole blood

REAGENTS AND BUFFERS

1. Cryostore (Sigma, Catalogue number C2874)
2. 1x DPBS (Invitrogen, Catalogue number 14190)
1. P3 Steril (Ecolab)

MATERIALS

1. Cryovials
2. 'Mr.Frosty' (Nalgene, Catalogue number C1562)
3. P10, P20, P200 and P1000 filter tips
4. Beaker for waste

EQUIPMENT

1. BSL3 facility
2. BSC2 safety cabinet
3. Bench top centrifuge
4. P10, P20, P200 and P1000 pipettes
5. -80 freezer

To samples in step 14, part 2 of the '*Peripheral Blood Immunophenotyping: Sample Preparation*' protocol:

1. Centrifuge the falcon tubes containing PBMCs for 5 minutes at 1600rpm at 4°C.
2. Pour off the supernatant into a waste beaker containing P3 Steril.
3. Calculate the volume of freezing medium required to reach a cell concentration of 1×10^7 cells/ml.
4. Working quickly, resuspend the cell pellet in ice-cold freezing media to a concentration of 1×10^7 cells/ml. Aliquot 1 ml of cells into appropriately labelled cryovials. Close lids tightly.
5. Place cryovials in a Mr Frosty. Freeze cells at -80°C, in a location that is not disturbed by repeated freezer access. Transfer to the liquid nitrogen day after.