Protocol: Cryopreservation of PBMC

Steps:

1. Freezing:
    1. Resuspend PBMC at $1x10^7$ viable lymphocytes per mL in 12.5% HSA (see stock preparation table).
    2. Slowly add an equal volume of 2X freezing medium (see stock preparation table), while gently swirling.
    3. Place cells on ice. Slowly pipet 1 mL per vial into cryovials on ice.
    4. Cap and place cryovials into a pre-cooled freezing container filled with 70% ethanol. Put at -80C for 24 h, then move to liquid nitrogen. Alternately, place cryovials into a controlled rate freezer, then transfer to liquid nitrogen.

2. Thawing:
    1. Warm cRPMI medium to 37C prior to use.
    2. Thaw cryovials (not more than 2-3 at a time) in a 37C water bath, removing to a biosafety hood when only a small bit of ice remains.
    3. Wipe the vials with 70% ethanol before opening.
    4. Slowly add 1 mL of warm cRPMI to the cells in the vial.
    5. Slowly transfer the diluted cells into a tube containing 8 mL of warm cRPMI.
    6. Centrifuge at 250 x G for 7 m. Decant the supernatant and gently flick the pellet. Gently resuspend in the desired volume of warm cRPMI.
    7. Count and determine cell viability. If necessary, wash again as above to concentrate.
    8. Rest cells for 6-18 h for use in functional assays.

Notes:

1. Cells should not be strongly agitated in the presence of DMSO, but gentle mixing is necessary to disperse the DMSO in the solution.

2. Excessive storage at -80C, or multiple transfers between -80C and liquid nitrogen should be avoided.

3. Wear gloves and eye protection when thawing cryovials, due to risk of explosion if liquid nitrogen has seeped into the vial.

4. Warm medium for diluting and washing thawed PBMC is very important to preserve viability.

5. Recovery should routinely be >60% and viability >80% using this procedure, and these can be used as acceptance criteria for functional assays with thawed cells.

6. A rest period at 37C will increase responses and cytokine staining intensity in functional assays, but is not beneficial for phenotypic staining.

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Stock Solution Recommendations:

Solution	**Stock Concentration**	**Preparation**
25% HSA	25% Human Serum Albumin (HSA) (Gemini #800-120)	Dissolve 25 g HSA in RPMI w/o serum, sterile filter. Store at 4C.
12.5% HSA	12.5% HSA	Mix 10 mL of 25% HSA with 10 mL of sterile RPMI w/o serum. Store at 4C.
2X Freezing Medium	10% HSA, 20% DMSO	Mix 10 mL of 25% HSA with 10 mL of sterile RPMI w/o serum and 5 mL of DMSO. Store at 4C.