

Protocol: Cryopreservation of PBMC

Steps:

- Freezing:
 - Resuspend PBMC at 1×10^7 viable lymphocytes per mL in 12.5% HSA (see stock preparation table).
 - Slowly add an equal volume of 2X freezing medium (see stock preparation table), while gently swirling.
 - Place cells on ice. Slowly pipet 1 mL per vial into cryovials on ice.
 - 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.
- Thawing:
 - Warm cRPMI medium to 37C prior to use.
 - 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.
 - Wipe the vials with 70% ethanol before opening.
 - Slowly add 1 mL of warm cRPMI to the cells in the vial.
 - Slowly transfer the diluted cells into a tube containing 8 mL of warm cRPMI.
 - 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.
 - Count and determine cell viability. If necessary, wash again as above to concentrate.
 - Rest cells for 6-18 h for use in functional assays.

Notes:

- Cells should not be strongly agitated in the presence of DMSO, but gentle mixing is necessary to disperse the DMSO in the solution.
- Excessive storage at -80C, or multiple transfers between -80C and liquid nitrogen should be avoided.
- Wear gloves and eye protection when thawing cryovials, due to risk of explosion if liquid nitrogen has seeped into the vial.
- Warm medium for diluting and washing thawed PBMC is very important to preserve viability.
- 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.
- 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.