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Patient PBMC collection and cryopreservation and cryorecovery

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We use this protocol and it's working

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Abstract

Isolation of PBMC from human blood using CPT tubes, cryopreservation and cryorecovery of cells

Troubleshooting

PBMC collection and cryopreservation

1 Collect 8 mL of blood per BD Vacutainer CPT Cell Preparation Tube with Sodium Citrate (BD Biosciences, 362761).

Invert tubes 8-10 times and centrifuged at room temperature at 1500*x g* for 20 minutes with no brake.

Using a 10mL stripette , transfer the PBMC enriched layer to a new 50-mL conical tube, being sure not to touch the ficoll layer, and add MACS buffer (PBS, 0.5% bovine serum albumin, 20 mM EDTA, pH 7.2) to a final volume of 50 mL, followed by centrifugation at 1800*x g* for 10 minutes at room temperature.

Following removal of the supernatant, resuspend PBMCs in 10mL MACS buffer and count using a hemocytometer with Trypan blue at a 1:20 dilution (10uL of cells mixed with 10uL of 1:20 Trypan blue solution).

Centrifuge PBMCs for 10 min at 300*x g* at 4°C.

Aspirate supernatant and gently resuspend cell pellet in cryopreservation media (RPMI 1640, with 20% FBS and 10% DMSO) at a final concentration of 1×7^{7} cells/mL in cryovials (Simport, T311-2).

Place cryovials in a room-temperature Mr. Frosty freezing container with isopropanol as per manufacturer's instructions and store at -80°C overnight.

Remove cryovials from freezing containers and immediately placed into liquid nitrogen for long-term storage.

Cryorecovery

2 For cryorecovery, retrieve PBMCs from liquid nitrogen, and thaw at 37°C in water bath.

Once thawed (roughly 1 minute with agitation) slowly add contents of one cryovial to 25mL 37°C filter sterilized complete culture media (RPMI 1640 media, 10% heat-inactivated FBS, 1mM Penicillin-Streptomycin) in 50mL falcon and pellet via centrifugation at 300*x g* for 10 min at room temperature.

Remove supernatant and resuspend cells in 37°C complete culture media for cell counting using Trypan blue.