PBMC isolation from Pall filters

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Solutions needed: PBS, PBS+1mM EDTA, Ficoll, RPMI media (optional: DMSO, FBS)

1. Push cells out of a Pall filter using a 60ml syringe, alternatingly filling the syringe with 50ml PBS and 50ml PBS + 1mM EDTA for a total volume of 200ml. Gently tap the filter periodically while pushing fluid through.

2. Pipet 50ml of blood into four 50ml conicals and spin at 2000rpm for 10 minutes.

3. Remove the white layer of cells off cell pellet and transfer to a new tube.

4. Add PBS to each tube to a volume of 15ml per tube and combine two tubes (30ml each in two conicals).

5. Add 15mL of Ficoll into a new 50mL conical and carefully overlay the 30mL of suspended cells onto the Ficoll. This is done by tilting the conical on its side and **very slowly** adding the suspended cells using a 25ml pipette. This should result in a clean line of separation between the Ficoll and the suspended cells. Repeat for all tubes.

6. Centrifuge at 2000rpm for 30 min with **NO** brakes. This will separate into PBS on top, PBMCs in the middle (visible as a white buffy coat) and Ficoll on the bottom.

7. Carefully pipette the white PBMC layer (at the interphase) into a 50mL conical tube. Bring volume up to 50mL with PBS.

8. Centrifuge at 2000rpm for 5 min with brakes **ON**.

9. Aspirate the supernatant. Flick the tube to dislodge the pellet. Resuspend the cells in 50mL of sterile PBS. Cells from all 50ml conicals can be combined into a final volume of 50ml for easier counting.

10. Count the cells (a 1:10 or higher dilution may be necessary depending on yields). Centrifuge at 2000rpm for 5 min with brakes **ON** to pellet the cells. Aspirate the supernatant, flick the tube to dislodge the pellet, and resuspend cells in RPMI media.

11. Alternatively, cells can be resuspended in freezing media (10% DMSO in FBS) at $5x10^6$ cells/mL, and aliquoted at 1mL per cryovial. Freeze cells in step down freezing container (Mr. Frosty) overnight, then transfer to liquid nitrogen tank.