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PBMC isolation

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Protocol status: Working

We use this protocol and it's working



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Abstract

Isolation of PBMCs from fresh whole human blood in Lithium Heparin blood tubes.

Materials

- SepMate Tubes – StemCell (1 per 17 mL blood)
- Divalent cation-free PBS (no calcium or magnesium) + 2% FBS, filtered
- Ficoll-Paque Plus (15 mL per 17 mL blood)
- Eppendorf tubes for serum
- Sterile pipet tips and sera pipets
- 50 mL falcons
- Haemocytometer
- FBS + 10% DMSO, filtered and chilled
- Cryovials
- Mr Frosty

Troubleshooting



Lymphocyte separation

- 1 - Start with everything at room temperature:
- 2 - Add 15 mL Ficoll-Paque Plus to each SepMate tube by pipetting directly into centre of plastic separator (try not to introduce a lot of air beneath the separator)
- 3 - Pool anti-coagulated blood tubes (3 × 9 mL Li Hep tubes) – should come to 27ml
- 4 - Dilute 1:1 in PBS + 2% FBS (now have ~54 mL diluted blood)
- 5 - Layer blood on top of Ficoll-Paque Plus by gently pipetting down the side of the SepMate tube (avoid pipetting directly above the small notches as this lets blood through to below the separator)
- 6 - Cap tightly and spin at 1200 x g, 10m, RT, accelerator and break on full

Washing

45m

- 7 - Cool PBS + 2% FBS in refrigerator and set centrifuge to cool (4C, though 10C should be sufficient)
- 8 - Pour interface off SepMate tube into new 50 mL falcon (2 SepMate tubes per falcon – if odd number of tubes, even out the volumes between falcons)
- 9 - Top cells up to 45 mL with chilled PBS + 2% FBS, mix well
- 10 - Centrifuge 900 x g, 10m in cooled centrifuge
- 11 - Meanwhile, centrifuge 5 mL serum tube in the same spin
- 12 - Discard supernatant and flick to dislodge pellet



- 13 - Resuspend cells in chilled PBS + 2% FBS and pool cells from each individual into 1 tube with 40 mL buffer
- 14 - Centrifuge 300 x g, 10m in cooled centrifuge
- 15 - Meanwhile, aliquot serum (avoid any red) and store aliquots in freezer (these do not contain any human cells)
- 16 - Discard supernatant and flick to dislodge pellet
- 17 - Resuspend cells in 25 mL chilled PBS + 2% FBS
- 18 - Take 10 uL aliquot and count on haemocytometer
- 19 - Centrifuge 300 x g, 10m in cooled centrifuge
- 20 - Discard supernatant and pipet off final drips, flick to dislodge pellet

Freezing

5m

- 21 - Resuspend cells in chilled 1-1.5 mL FBS + 10% DMSO (if more than 15 million cells, make multiple aliquots) – Freeze in ~5million cell aliquots
- 22 - Rapidly move cryovials to Mr Frosty and put in -80C Freezer