PBMC isolation

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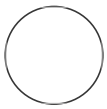
Morgan



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Protocol status: Working
We use this protocol and it's working

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DISCLAIMER

ABSTRACT

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

PROTOCOL integer ID: 59233

Keywords: PBMC, SepMate, Whole blood

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

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 x 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