Mar 08, 2022

PBMC isolation

DOI
dx.doi.org/10.17504/protocols.io.b539q8r6

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Protocol Citation: Michael Morgan 2022. PBMC isolation . **protocols.io https://dx.doi.org/10.17504/protocols.io.b539q8r6**

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

Created: March 08, 2022

Last Modified: March 08, 2022

Protocol Integer ID: 59233

Keywords: PBMC, SepMate, Whole blood, lithium heparin blood tube, isolation of pbmc, pbmc isolation, pbmc, fresh whole human blood

Disclaimer

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