



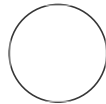
MAR 08, 2022

🌐 PBMC isolation

Michael
Morgan^{1,2}

¹Cancer Research UK - Cambridge Institute; ²University of Cambridge

Morgan



Michael Morgan

OPEN  ACCESS



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

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

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Mar 08, 2022

Last Modified: Mar 08, 2022

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](#) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](#), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

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

PROTOCOL integer ID:
59233

MATERIALS

Keywords: PBMC, SepMate,
Whole blood

- 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

- 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

