OCT 29, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6zyhf7w

External link:
<https://doi.org/10.1371/journal.pone.0224276>

Protocol Citation: John Davis Coakley 2019. Peripheral Blood Mononuclear Cells Preparation. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.6zyhf7w>

MANUSCRIPT CITATION:
 Coakley JD, Breen EP, Moreno-Olivera A, Al-Harbi AI, Melo AM, O'Connell B, McManus R, Doherty DG, Ryan T (2019) Dysregulated T helper type 1 (Th1) and Th17 responses in elderly hospitalised patients with infection and sepsis. PLoS ONE 14(10): e0224276. doi: [10.1371/journal.pone.0224276](https://doi.org/10.1371/journal.pone.0224276)

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Protocol status: Working

Peripheral Blood Mononuclear Cells Preparation

PLOS One

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ABSTRACT

How to prepare PBMCs for Stimulation experiment

GUIDELINES

Sterile conditions, biosafety cabinet

MATERIALS

15mls whole blood (fresh)
 1% FCS= Foetal Calf Serum (Phosphate buffered saline with 1% foetal calf serum)
 RPMI = RPMI 1640 containing GlutaMAX, 10% HyClone FBS, 50 mg/mL streptomycin, 50 U/mL penicillin, 2.5 µg/ml amphotericin B - fungizone, and 25 mM HEPES
 Lymphoprep™ (Axis-Shield, Dundee, UK)
 ethidium bromide/acridine orange.

Created: Sep 01, 2019

Last Modified: Oct 29, 2019

PROTOCOL integer ID:
27416

- 1 Dilute whole blood 1:1 with 1% FCS HBSS Medium
- 2 Layer this diluted blood onto 10mls Lymphoprep™
- 3 Centrifuge for 25 minutes at 400G with acceleration and brake off
- 4 Discard the plasma and obtain the buffy coat
- 5 Top up buffy coat with 1% FCS, vortex and Centrifuge for 8 minutes at 1500RPM with brake on. Discard supernatant
- 6 Top up with 1% FCS, vortex and centrifuge for 8 minutes at 1500RPM with brake on. Discard supernatant.
- 7 Resuspend in 4-5mls of RPMI, and vortex.
- 8 Count live cells with haemocytometer slide after staining with ethidium bromide and acridine

orange solution to detect dead and live cells, respectively