Peripheral Blood Mononuclear Cells Preparation

PLOS One

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1 Works for me

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ABSTRACT

How to prepare PBMCs for Stimulation experiment

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0224276

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

GUIDELINES

Sterile conditions, biosafety cabinet

MATERIALS TEXT

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.

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