Peripheral blood mononuclear cell isolation and stimulation

Version 4

Dorota Kmiec

Abstract

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Protocol

Step 1.
Bring all reagents and the centrifuge (Eppendorf 5810R) to a room temperature.

Step 2.
Dilute buffy coat (Deutsches Rotes Kreuz, DRK) 1:4 with DPBS (Gibco).

Step 3.
Add 20ml of Biocoll separating solution (Merck, cat# L6115) into 50 ml plastic tube.

Step 4.
Slowly pipette 20ml of diluted buffy coat over Biocoll solution without mixing the layers.

Step 5.
Centrifuge at 1200 RCF for 20 min with the brake switched off.

Step 6.
Transfer the white interface containing PBMCs to a new 50ml tube.

Step 7.
Add DPBS up to the total volume of 40ml and centrifuge at 1400rpm for 5min.

Step 8.
Remove the supernatant and resuspend the pellet in 40ml of DPBS.

Step 9.
Centrifuge again at 1400rpm for 5min.

Step 10.
Remove the supernatant and resuspend cell pellet in 10ml of RPMI1640 supplemented with 2 mM L-glutamine, 100 µg/ml streptomycin, 100 units/ml penicillin, 10 % (v/v) heat inactivated FCS (Gibco).

Step 11.
Determine cell concentration using haemocytometer.

Step 12.
If PBMCs are to be infected with SIV or HIV follow steps 13-14.

Step 13.
Adjust cell concentration to 2.000.000 cells/ml by adding more medium.

Step 14.

Add 1 µg/ml PHA (Murex) and 10 ng/ml IL-2 (Sigma-Ark) for stimulation and incubate for 3 days at

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37°C, 5%CO2.

Step 15.

Stimulated PBMCs form large clumps. Depending on the blood donor, the number of viable cells can decrease up to 50% after 3 days. Some cells might adhere to the walls of the flask and (if necessary) can be removed by gentle pipetting.

Step 16.

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