CD4+ T cell isolation and stimulation Version 2

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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 50µl of RosetteSep™ Human CD4+ T Cell Enrichment Cocktail (Stemcell) per 1ml of the diluted buffy coat.

Step 4.
Incubate for 20min at room temperature.

Step 5.
Add 20ml of Biocoll separating solution (Biochrom, cat# L6115) into 50 ml plastic tube.

Step 6.
Slowly pipette 20ml of diluted buffy coat over Biocoll solution without mixing the layers.

Step 7.
Centrifuge at 1200 RCF for 20 min with the brake switched off.

Step 8.
Transfer the white interface containing CD4+ T cells to a new 50ml tube.

Step 9.
Add DPBS up to the total volume of 40ml and centrifuge at 1400rpm for 5min.

Step 10.
Remove the supernatant and resuspend the pellet in 40ml of DPBS.

Step 11.
Centrifuge again at 1400rpm for 5min.

Step 12.
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 13.
Determine cell concentration using haemocytometer.

Step 14.
Optional: determine isolated cell purity by staining for CD4 and CD11c surface receptors and flow cytometric analysis. Suggested antibodies: anti-CD4-PerCP (cat# 550631, BD Biosciences) and anti-

CD11c-FITC (cat#ab22540, abcam). CD4+ T cells are CD4+ and CD11c - .

Step 15.
If cells are to be infected with SIV or HIV follow steps 16-17.

Step 16.
Adjust cell concentration to 2.000.000 cells/ml by adding more medium.

Step 17.
Add 1 µg/ml PHA (Murex) and 10 ng/ml IL-2 (Sigma-Ark) for stimulation.

Step 18.

Incubate for 3 days at 37°C, 5%CO2. Stimulated cells should form large clumps.