VERSION 2

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CD4+ T cell isolation and stimulation V.2

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- 1 Bring all reagents and the centrifuge (Eppendorf 5810R) to a room temperature.
- 2 Dilute buffy coat (Deutsches Rotes Kreuz, DRK) 1:4 with DPBS (Gibco).
- 3 Add 50µl of RosetteSep™ Human CD4+ T Cell Enrichment Cocktail (Stemcell) per 1ml of the diluted buffy coat.
- 4 Incubate for 20min at room temperature.
- 5 Add 20ml of Biocoll separating solution (Biochrom, cat# L6115) into 50 ml plastic tube.
- 6 Slowly pipette 20ml of diluted buffy coat over Biocoll solution without mixing the layers.

- 7** Centrifuge at 1200 RCF for 20 min with the brake switched off.
- 8** Transfer the white interface containing CD4+ T cells to a new 50ml tube.
- 9** Add DPBS up to the total volume of 40ml and centrifuge at 1400rpm for 5min.
- 10** Remove the supernatant and resuspend the pellet in 40ml of DPBS.
- 11** Centrifuge again at 1400rpm for 5min.
- 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).
- 13** Determine cell concentration using haemocytometer.
- 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 - .

- 15** If cells are to be infected with SIV or HIV follow steps 16-17.
- 16** Adjust cell concentration to 2.000.000 cells/ml by adding more medium.
- 17** Add 1 µg/ml PHA (Murex) and 10 ng/ml IL-2 (Sigma-Ark) for stimulation.
- 18** Incubate for 3 days at 37°C, 5%CO₂. Stimulated cells should form large clumps.