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Peripheral blood mononuclear cell isolation and stimulation V.4

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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 20ml of Biocoll separating solution (Merck, cat# L6115) into 50 ml plastic tube.
- 4 Slowly pipette 20ml of diluted buffy coat over Biocoll solution without mixing the layers.
- 5 Centrifuge at 1200 RCF for 20 min with the brake switched off.
- 6 Transfer the white interface containing PBMCs to a new 50ml tube.

- 7** Add DPBS up to the total volume of 40ml and centrifuge at 1400rpm for 5min.
- 8** Remove the supernatant and resuspend the pellet in 40ml of DPBS.
- 9** Centrifuge again at 1400rpm for 5min.
- 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).
- 11** Determine cell concentration using haemocytometer.
- 12** If PBMCs are to be infected with SIV or HIV follow steps 13-14.
- 13** Adjust cell concentration to 2.000.000 cells/ml by adding more medium.
- 14** Add 1 µg/ml PHA (Murex) and 10 ng/ml IL-2 (Sigma-Ark) for stimulation and incubate for 3 days at 37°C, 5%CO₂.

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.

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