SOP: PBMC Preparation, Version 3, 2016_03_29

REAGENTS:

I. EDTA tubes, 9 mL or 10 mL blood each
II. Ficoll, density 1.077g/mL (Biochrom Cat. No. L6115), 4°C, store in the dark
III. PBS Dulbecco w/o Mg^{++} Ca^{++} (Biochrom L1825), 4°C
IV. +10% heat inactivated FCS (56°C water bath for 20 min), 4°C
V. DMSO (Dimethylsulfoxid, e.g. Sigma, # 41640)

Transfer the blood

1. Pour the EDTA blood from 2-3 tubes (25-30 mL) from the blood sample tubes into one 50 mL Falcon tube and centrifuge at 1,500g for 10 minutes at 4°C.
2. Transfer the plasma (~ 6 mL) of the centrifuged Falcon tubes into 6 cryo tubes (1 mL each), store at -80°C.
3. Fill up the Falcon tube that was used for plasma collection to 50 mL with PBS.
4. Collect the blood of 2-3 EDTA tubes in a 50 mL Falcon tube and fill up to 50 mL with PBS. This will result in 2 x 50 mL Falcon tubes totally.

Ficoll

5. Fill 15 mL of Ficoll into 3 50 mL Falcon tubes each and slowly overlay with ~35 mL of diluted EDTA blood (see 4).
6. Centrifuge at 1,000g for 17 minutes, at 21°C, NO BRAKE!!!

Isolation of the lymphocytes

7. Remove the lymphocyte ring with a 10 mL short-pipette and transfer into a new 50 ml Falcon tube, fill up to 50 mL with PBS.
8. Centrifuge at 500g 20 minutes, 4°C, max. BRAKE!, discard the supernatant (SN).
9. Resuspend the pellets of the Falcon tubes in 50 mL PBS, count cells, and wash as in (8) at 500g max. BRAKE!, for 10 minutes at 4°C, discard the SN.

Counting and freezing

10. Collect all cells in 90% heat inactivated FCS + 10%DMSO at concentrations of $5x10^6$ PBMCs/mL (4 tubes with 1 mL each), $10x10^6$ PBMCs/mL (4 tubes with 1mL each), and the rest at $20x10^6$ PBMCs/mL in as many tubes as required.
11. Slowly freeze down with cryobox containing isopropanol. Put cryobox in -80°C and transfer samples into liquid nitrogen the next day.