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Isolation and storage of PBMCs from human peripheral blood samples

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ABSTRACT

This protocol descried detailed steps on how to isolate and store PBMCs from human peripheral blood samples. By following this protocol, you can isolate and store PBMCs for up to 1 year. Then you can perform antibody staining on samples of different timepoints/batches for flow cytometry analysis once.

Isolation and storage of PBMCs from human peripheral bloo..

1 Collect 2 tubes of 10ml of blood in EDTA tubes and transport to the laboratory in an ice box.

2 Spin samples at 1600 rpm (2500 x G) for 10 minutes in collection tubes.

3 Collect plasma and transfer to 5ml tubes.

4 Spin plasma at 1800 rpm for 10 minutes and transfer/aliquot to 1ml labelled cryovials and store in -80 °C freezer for cytokines analyses. (Label cryovials with project name, subject ID number, date and time of sample collected, and initial of who processed the samples).

5 Dilute remaining blood by adding equal volume (of collected plasma samples, around 4ml) of PBS per tube and mix well.

6 Add 5ml of Ficoll to a sterile 15ml conical tube and overlay diluted blood (always use a 1:2 ratio of Ficoll to blood)-one tube per tube of blood.

7 Spin samples at 2200 rpm for 25 minutes at room temperature with no brake or acceleration.

8 Collect interface (between PBS and Ficoll) and transfer to a new 15mL conical tube.

9 Wash the cells with 12ml 1XPBS and spin samples at 1700 rpm for 8 minutes.

10 10. Perform second wash by discarding supernatant and bringing the volume up to 15ml with PBS then spin samples at 1500 rpm for 5 minutes

11 11. Discard supernatant and completely break up pellet, resuspended in 10 mL PBS and count cells using cell counter (Countess, Invitrogen), record total number of cells in each sample.

12 12. Freeze the resuspend cells in cold freezing media (90% FBS, 10% DMSO) transfer 1 ml to labeled cryovials (label as detailed in plasma samples) and place in Mr. Frosty which is filled with 100 Ethanol.

13 13. Freeze at least 4x10^6 cells per vial and aim for at least 2 vials per sample. Record number of vials to be frozen.

14 14. Store Mr. Frosty in -80 °C freezer overnight and transfer cryovials to liquid nitrogen the following morning for future Flow cytometry analysis.

15 15. Both -80 °C freezer and liquid nitrogen tank need to be locked to protect these samples according to approved IRB protocol.