Columbia University Medical Center

Columbia Center for Translational Immunology

Columbia University College of Physicians and Surgeons
650 West 168th Street
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Blood Processing

A. Reagents Needed

DNAzol
PBS Sterile solution 1X
Ficoll
ACK Lysing Buffer
Trypan Blue
Chilled Filtered 0.22μm 100% FCS
Chilled Filtered 0.22μm 80% FCS 20% DMSO

B. Objective: to separate and store DNA, Blood Plasma and PBMCs.

C. Description

DNA

1. Invert the blood tube 2-3 times to ensure homogenous mixture.
2. Using a sterile pipet, take out 1ml of whole blood and transfer into 15ml falcon tube.
3. Mix whole blood with 2ml of DNAzol.
4. Aliquot Blood-DNAzol mixture (1.5ml/vial) into two labeled cryovials.
5. Store vials immediately at -80° C.

Plasma

1. Centrifuge blood samples at 1500RPM for 10 minutes at Room temperature (RT).
2. Reserve spun blood.
3. Harvest supernatant (Plasma) into separate 15ml falcon tube.
4. Re-spin the plasma at 2500RPM for 5 minutes at RT.
5. Aliquot supernatant into 5 labeled round-bottom cryovials and store in -80° C

Isolating PBMC

6. Add 5ml of Ficoll to each 15ml tube (or 10 of Ficoll to the 50ml Falcon tube).
7. Re-suspend the whole blood to two times its original volume with sterile PBS.
8. Homogenize by pipetting up and down.
9. Use a 15ml Falcon tube if the amount of blood is below 4.5ml, if above use 50ml tubes.

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10. Tip tube of Ficoll almost horizontally and add diluted blood EXTREMELY SLOWLY right above the Ficoll. Do not let the blood and Ficoll mix, Ficoll and blood layers should remain separate.
11. Centrifuge the Ficoll/blood at 2500RPM for 15 minutes at RT. **IMPORTANT: set deceleration to "0" and acceleration to "3"**
12. Harvest the cell ring (PBMC) located above the Ficoll in a circular movement.
13. Transfer cells into a second 15ml or 50ml tube filled with PBS (5ml for 15ml tube/15ml for 50ml tube).
14. Spin cells at 1500RPM for 5 minutes (spin 10min if using 50ml tube) at RT.
15. Discard supernatant and gently tap bottom of tube to re-suspend pellet.
16. Re-suspend cells again with PBS 1X (if pellet looks red, re-suspend cells in 3-5ml of ACK buffer to lyse remaining RBC's, wash with PBS after 5 minutes) Transfer the cell suspension into a 15ml tube, if originally in 50ml tube.
17. Spin cells again at 1500RPM for 5 min at RT.
18. Discard supernatant, re-suspend in 5ml PBS.

Cell Counting

19. Pipet cell suspension up and down to homogenize. Take a drop from the center.
20. Pipette 10µl of Trypan Blue into a test tube, add 10µl of cell suspension and mix thoroughly. Add 10µl of cell/dye mixture to hemocytometer.
21. Count 2 quadrants of cells, take the average, and apply cell concentration formula: #of cells x 2 x total volume of cell suspension x 10^4 (e.g. 40 cells counted in 5ml of PBS→ 40 x 2 x 5 x 10^4 = 4 x 10^6 cells total). Count at least 50 cells, if less than 50 cells in 2 quadrants, count all 4 quadrants and average.

Cryopreservation

22. Determine number of round-bottom cryovials to make as follows:
    - Less than 3 x 10^6 cells: 2 cryovials.
    - 3-4 x 10^6 cells: 3 cryovials.
    - 4-5 x 10^6 cells: 4 cryovials.
    - Continue up to 5 cryovials max post-transplant; 15 max pre-transplant (amount may vary depending on study/organ type).
23. FREEZE ONLY 10 or less aliquots at a time.
24. Always keep 0.22µm filtered FCS and FCS/DMSO in ice.
25. After counting cells, centrifuge cell suspension at 1500RPM for 5 minutes at RT.
26. Label appropriate number of cryovials and chill on ice.
27. Discard supernatant and re-suspend cells in 0.22µm filtered FCS (0.5ml/vial to be made).

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28. Distribute 0.5ml of cell re-suspended in ice-cold FCS into each chilled cryovial.
29. Add 0.5ml ice cold 0.22μm filtered 80% FCS/ 20% DMSO to each cryovial of cells to make a 1:1 solution of FCS/FCS-DMSO
30. Recap and turn vial containing cells and preservative upside down several times to homogenize and quickly store in -80° C.
31. Long-term storage of frozen cells in Li N_2.