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Protocol status: Working
 We use this protocol and it's working

Created: Jul 21, 2021

Isolation of Nucleated Cells from Whole Blood

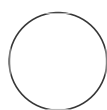
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ABSTRACT

This protocol describes a method for the isolation of pan-lymphocytes and pan-myeloid cells from human whole blood. By providing defined media formulations, volumes at each step, and a defined dilution factor for density centrifugation, it yields consistent single-cell suspensions across samples.

ATTACHMENTS

[dzhmbk587.pdf](#)

MATERIALS









Materials:

- Dulbeccos phosphate-buffered saline (DPBS) Gibco - Thermo Fischer Catalog #14190144
- Penicillin-Streptomycin-Glutamine (100X) Thermo Fisher Catalog #10378016
- Thermo Scientific™ Nunc™ 50mL Conical Sterile Polypropylene Centrifuge Tubes Fisher Scientific Catalog #12-565-271
- Gibco™ IMDM (Iscoves Modified Dulbeccos Medium) Fisher Scientific Catalog #12-440-053
- Gibco™ Fetal Bovine Serum qualified Australia Fisher Scientific Catalog #10-099-141
- UltraPure™ 0.5 M EDTA pH 8.0 Thermo Fisher Scientific Catalog #15575020

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PROTOCOL integer ID: 51742

Keywords: Blood, CD45, Lymphocytes, Myeloid, Isolation, Density gradient, Ficoll, Immune, 10x, scRNAseq, Flow cytometry, WBC, Leukocyte, Single cell suspension, T cell

-  Ficoll-Paque™ PLUS Media Fisher Scientific Catalog #45-001-749
-  Mr. Frosty™ Freezing Container Fisher Scientific Catalog #5100-0001
-  CryoStor CS10 100ML Fisher Scientific Catalog #NC9930384
-  Corning™ Externally Threaded Cryogenic Vials Fisher Scientific Catalog #09-761-71
-  5mL Falcon™ Round-Bottom Polypropylene Test Tubes Fisher Scientific Catalog #14-959-11A
-  Solution 13 AO – DAPI Chemometec Catalog #910-3013
-  NC-Slide A8™ box with 25 Slides Chemometec Catalog #942-0003
-  Falcon™ Plastic Disposable Transfer Pipets Fisher Scientific Catalog #1368050

Equipment:

- Centrifuge
- Cell Counter - NC-3000

Preparing Mediums and Buffers

1 Create the following **IMDM-FBS-PSQ Media** in a 500mL bottle of IMDM by using the table below:

A	B	C	D
Component	Volume (mL)	Starting Conc.	Final Conc.*
IMDM	500	-	-
Penicillin-Streptomycin-Glutamine	5	100X	1X

A	B	C	D
FBS	50	100%	10%

Table 1.

*Final Concentration is approximate.

- 2 Create the following **DPBS-FBS-EDTA Solution** in a bottle of DPBS by using the table below:

A	B	C	D
Component	Volume (mL)	Starting Conc.	Final Conc.*
DPBS	500	-	-
FBS	25	100%	5%
EDTA	1	0.5M	1mM


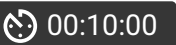

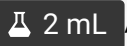
Table 2.

*Final Concentration is approximate.

Preparation of Blood

10m

- 3 Record the total volume of whole blood to be processed.
_____mL

- 4 Spin the whole blood  400 x g for  00:10:00 in the anti-coagulant tubes, remove the plasma  10m layer, and distribute to cryovials – up to  2 mL /vial.

Record the total volume of plasma: _____ mL and the number of vials: _____.

- 5 Replace the plasma volume removed from the whole blood with DPBS-FBS-EDTA Solution.

- 6 Divide the whole blood into  10 mL aliquots and distribute to separate  50 mL tubes.

7 Dilute the whole blood using 4 volumes or  40 mL DPBS-FBS-EDTA Solution; invert to mix.







Note


NOTE: This is the optimum dilution to maximize cell recovery.





Ficoll-Paque







40m






8 Layer the blood/DPBS-FBS-EDTA Solution mixture from the  50 mL tubes  25 mL at a time in separate  50 mL tubes on top of  15 mL of Ficoll-Paque Media PLUS.


Note

NOTE: For any remaining volume, add DPBS-FBS-EDTA Solution to bring the volume to  25 mL, and layer as described in this step.

9 Spin for  00:20:00,  1200 x g at  20 °C with 4 acceleration and 0 brake, evenly distribute  20m tubes across the entire rotor to prevent wobbling (use all four buckets if possible as opposed to just two).

10 Remove the mononuclear cell layer from each tube with a transfer pipet to  50 mL tubes -  10m mononuclear layers may be combined at this step to reduce the number of tubes to spin. Add cold DPBS-FBS-EDTA Solution to a final volume of  50 mL and centrifuge the cell suspensions for  00:10:00 at  400 x g,  4 °C.





11 Remove the supernatant and re-suspend the cell pellet in  50 mL cold DPBS-FBS-EDTA Solution  10m centrifuge the cell suspension for  00:10:00 at  120 x g,  4 °C.

12 Remove the supernatant and re-suspend the cell pellet in cold  10 mL IMDM-FBS-PSQ Media.

Cell Count

- 13 Count cells, and viability by using the NC-3000 cell counter. Calculate total viable cells and record below:
cell number: _____ cells/mL, _____ % viable
final volume: _____ mL
*cell number (cells/mL) * viability(%) * final volume(mL) = total viable cells*
Total Viable Cells: _____

Freeze-down and QC

- 14 (Optional QC) Aliquot 2×10^6 cells to a  5 mL Falcon tube and place on ice for subsequent flow cytometric analysis.
- 15 Aliquot cells for analysis or experimentation, and then freeze down remaining cells in up to 2×10^7 aliquots using Cryostor CS10 Medium, a Mr. Frosty, and a  -80 °C freezer ( 1 mL -  1.5 mL aliquots, round down to the nearest 20 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.