Isolation of Nucleated Cells from Whole Blood

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1. 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.

2. Materials

Dulbecco's Phosphate Buffered Saline (DPBS) (Fisher Scientific, Cat. No.: 14-190-144)

Penicillin-Streptomycin-Glutamine (100X) (Fisher Scientific, Cat. No.: 10-378-016)

50mL Centrifuge Tube (Fisher Scientific, Cat. No.: 12-565-271)

Iscove's Modified Dulbecco's Medium (IMDM) (Fisher Scientific, Cat. No.: 12-440-053)

Fetal Bovine Serum (FBS) (Fisher Scientific, Cat. No.: 10-099-14)

EDTA 0.5M pH 8.0 (Fisher Scientific, Cat. No.: 15-575-020)

Ficoll-Paque™ PLUS Media (Fisher Scientific, Cat. No.: 45-001-749)

Mr. Frosty (Fisher Scientific, Cat. No.: 51000001)

Cryostor CS10 (Fisher Scientific, Cat. No.: NC9930384)

Cryogenic Vials (Fisher Scientific, Cat. No.: 09-761-71)

5mL Falcon™ Round-Bottom Polypropylene Tubes (Fisher Scientific, Cat. No.: 14-959-11A)

Solution 13 AO/DAPI (Chemometec, Cat. No.: 910-3013)

NC-Slide A8 (Chemometec, Cat. No.: 942-0003)

Falcon™ Plastic Disposable Transfer Pipets (Fisher Scientific, Cat. No.: 13-680-50)

3. **Equipment**

Centrifuge

Cell Counter - NC-3000

4. **Protocol**

4.1. **Preparing Mediums and Buffers**

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

Component	*Volume (mL)*	*Starting Conc.*	*Final Conc.**
IMDM	500		-
Penicillin-Streptomycin-Glutamine	5	100X	1X
FBS	50	100%	10%

Table 1.
**Final Concentration is approximate.*

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

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

Table 2.
**Final Concentration is approximate.*

4.2. **Preparation of Blood**

4.2.1. Record the total volume of whole blood to be processed.

_____mL

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

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

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

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

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

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

4.3. **Ficoll-Paque**

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

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

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

4.3.3. Remove the mononuclear cell layer from each tube with a transfer pipet to 50mL tubes - 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 50mL and centrifuge the cell suspensions for 10 minutes at 400 x g, 4°C.

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

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

4.4. Cell Count

4.4.1. 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

$$cell\ number\left(\frac{cells}{mL}\right) * viability(\%) * final\ volume(mL) = total\ viable\ cells$$

Total Viable Cells: _____

4.5. Freeze-down and QC

4.5.1. **(Optional QC)** Aliquot 2 x 10^6 cells to a 5mL Falcon tube and place on ice for subsequent flow cytometric analysis.

4.5.2. Aliquot cells for analysis or experimentation, and then freeze down remaining cells in up to 2 x 10^7 aliquots using Cryostor CS10 Medium, a Mr. Frosty, and a -80°C freezer (1-1.5mL aliquots, round down to the nearest 20 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.