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

Created: Jul 20, 2021

Isolation of Nucleated Cells from Bone Marrow Aspirate

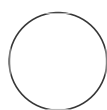
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ABSTRACT

This protocol describes a method for the isolation of pan-lymphocytes, pan-myeloid cells, and progenitors from human bone marrow aspirate. 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

[dzhkbk587.pdf](#)

MATERIALS








Materials:

- DPBS no calcium no magnesium Thermo Fisher Scientific 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: 51732

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

-  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

Equipment:

- Centrifuge
- Cell Counter - NC-3000

Preparing Mediums and Buffers

- 1 Create the following IMDM-FBS-PSQ Media in a  500 mL 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
FBS	50	100%	10%

Table 1.

*Final Concentration is approximate.

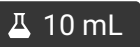


- 2 Create the following DPBS-FBS-EDTA Solution in a bottle of DPBS by using the table below:

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 Bone Marrow

- 3 Record the total volume of bone marrow to be processed.
_____mL.
- 4 Divide the bone marrow into  10 mL aliquots and distribute to separate  50 mL tubes.
- 5 Dilute the bone marrow using 4 volumes or  40 mL of DPBS-FBS-EDTA Solution; invert to mix.






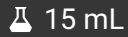
Note

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


Ficoll-Paque


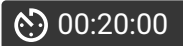
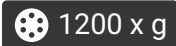


40m


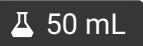




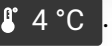
- 6 Layer the bone marrow/ DPBS-FBS-EDTA Solution mixture from the  50 mL tubes  25 mL at a




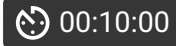

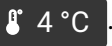
time in separate  50 mL tubes on top of  15 mL of Ficoll-Paque Media PLUS.


Note

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

7  Spin for ,  at  with 4 acceleration and 0 brake, evenly distribute  tubes across the entire rotor to prevent wobbling (use all four buckets if possible as opposed to just two).

8  Remove the mononuclear cell layer from each tube with a transfer pipet to  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  and centrifuge the cell suspensions for  at , .




9  Remove the supernatant and re-suspend the cell pellet in  cold DPBS-FBS-EDTA Solution  centrifuge the cell suspension for  at , .

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

Cell Count

11 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

- 12 (Optional QC) Aliquot 2×10^6 cells to a 5mL Falcon tube and place on ice for subsequent flow cytometric analysis.
- 13 Aliquot cells for analysis or experimentation, and then freeze down remaining in up to 3×10^7 aliquots using Cryostor CS10 Medium, a Mr. Frosty, and a  $-80\text{ }^{\circ}\text{C}$ freezer ( 1 mL -  1.5 mL aliquots, round down to the nearest 30 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.