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

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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

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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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- 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 25 mL, and layer as described in this step.

7 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).

8 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.

9 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.

10 Remove the supernatant and re-suspend the cell pellet in cold 10 mL 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 x 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 x 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 30 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.