Isolation of Nucleated Cells from Whole Blood

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NOV 09, 2023

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bwr6pd9e

Protocol Citation: Steven B. Wells, Peter A. Szabo, Nora Lam 2023. Isolation of Nucleated Cells from Whole Blood. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bwr6pd9e

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

Created: Jul 21, 2021

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**

Last Modified: Nov 09, 2023

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 x 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 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 20 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.