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

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Preparation of Single Cell Suspension from Human Lymph Node Tissue

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ABSTRACT

This protocol describes a method for the isolation of pan-lymphocytes, pan-myeloid cells, and progenitors from human lymph node tissue. 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:

- Fisherbrand™ Sterile Syringes for Single Use **Fisher Scientific Catalog #14955459**
- 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**

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PROTOCOL integer ID: 51746

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

- 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**
- Thomas ScientificSupplier Diversity Partner Cell Strainer 100um Yellow Sterile Individually Wrap **Fisher Scientific Catalog #50-146-1428**
- Ficoll-Paque™ PLUS Media **Fisher Scientific Catalog #45-001-749**
- Collagenase D **Sigma Aldrich Catalog #11088882001**
- DNASE 1 100MG **Fisher Scientific Catalog #NC9709009**
- 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:

- Multi-Axle-Rotating Mixer/Shaker with Temperature Control
- Centrifuge
- Cell Counter - NC-3000
- Surgical scissors
- Scale

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 without calcium and magnesium 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.

Tissue Dissociation

32m

3 Clean lymph nodes of fat and connective tissue post dissection, record the site below.

_____

4 Add up to 2 ± 10% grams of cleaned lymph node tissue to per 50 mL centrifuge tube – record the total weight below.

_____g

Note

NOTE: Going beyond the 2 grams of tissue per tube reduces the efficacy of the enzymatic digest and lowers yields.

5 Add 5 mL of room temperature IMDM (NO ADDITIVES! Just the base media formulation) to each tube and use a scissors to chop the tissue into a fine “mash”.

6 Add 35 mL of room temperature IMDM (NO ADDITIVES! Just the base media formulation) and s 30m in 0.400 mL of Collagenase D, and 0.400 mL of DNAse to the tube to begin the enzymatic digestion. Place on a shaker or rotator for 00:30:00 at 37 °C.

7 After digestion, add 0.500 mL of EDTA [M] 0.5 Molarity (M) pH 8.0 to the digested cell suspensions and incubate for 00:02:00 at 20 °C. 2m

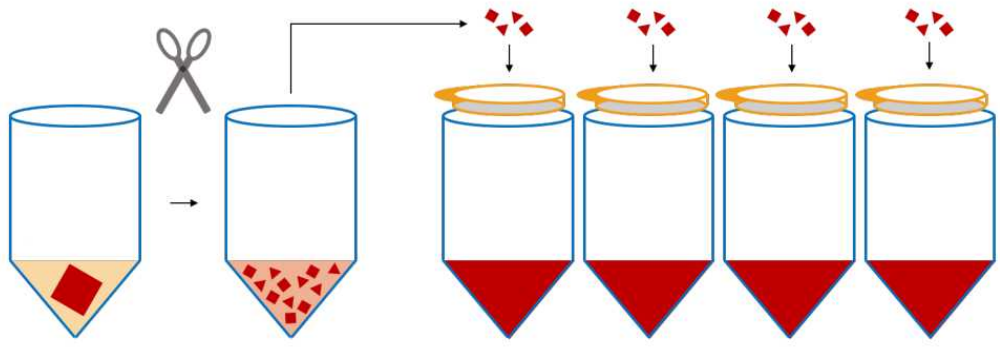

Figure 1. Steps 4.2.3 through 4.2.7.

8 Distribute and filter the mash of tissue over [M] 100 micromolar (µM) cell strainers above 50 mL tubes (about 4 filters/2 grams of tissue).

Note

NOTE: Cell yields and ease of pushing through the filter are increased by using multiple filters/gram of tissue, default to using more filters to decrease processing time, and increase yields.

9 Apply pressure with the black rubber bottom or the plastic end of a 10 mL syringe plunger to any remaining, partially digested tissue on the cell strainers, and intermittently wash through with DPBS-FBS-EDTA Solution from a transfer pipet – the aim is to push and wash through the tissue until only light pink/white/grey connective tissue remains. When finished, combine the tubes of cell suspension and proceed to the next section.

Ficoll-Paque

50m

10 Centrifuge the cell suspensions for 00:10:00 at 400 x g at 20 °C. 10m

11 Remove the supernatants and combine the cell pellets down to a single 50 mL tube, top to 50 mL with Room temperature IMDM-FBS-PSQ Media, spike in 0.500 mL of EDTA [M] 0.5 Molarity (M) pH 8.0.

12 Filter the cell suspension through a [M] 100 micromolar (µM) cell strainer.

13 In two 50 mL tubes, layer 25 mL of cell suspension on top of 15 mL of Ficoll-Paque Media PLUS.

14 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).

15 Remove the mononuclear cell layer from both tubes with a transfer pipet and combine in one 50 mL 10m tube. Add cold DPBS-FBS-EDTA Solution to a final volume of 50 mL and centrifuge the cell suspension for 00:10:00 at 400 x g, 4 °C.

16 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.

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

Cell Count

18 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: _____

Analysis and Freeze-down

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

20 Aliquot cells for analysis or experimentation, and then freeze down cells 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 20 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.