Preparation of Single Cell Suspension from Human Spleen Tissue

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

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ABSTRACT

This protocol describes a method for the isolation of pan-lymphocytes, pan-myeloid cells, and progenitors from human spleen 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

dzhhbk587.pdf

MATERIALS

Materials:

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

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- UltraPure™ 0.5M EDTA pH 8.0 **Fisher Scientific Catalog #15575020**
- 100µM cell strainer (Fisher Scientific, Cat. No.: 50-146-1428)
- 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
- Surgical scissors
- Scale

Preparing Medium and Buffer

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

3 Add 2 ± 10% grams of spleen tissue to a 50 mL centrifuge tube and record below.

_____g

4 Add 5 mL of DPBS-FBS-EDTA Solution to the spleen tissue and use a scissors to chop the tissue into a fine “mash”.

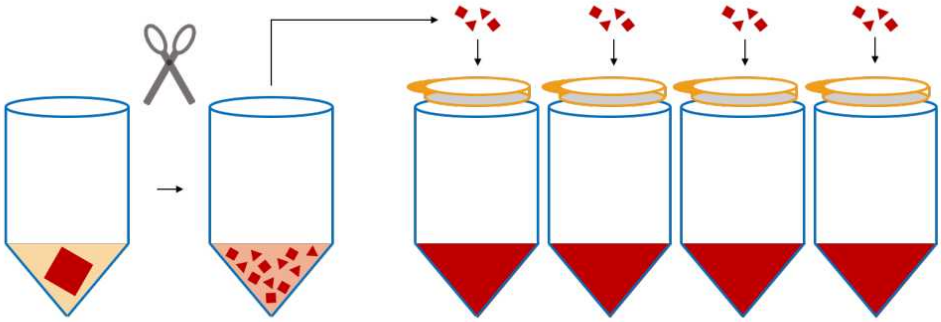

Figure 1. Steps 4.2.2 through 4.2.4.

5 Add 35 mL of DPBS-FBS-EDTA Solution to the mash of tissue, and distrubute and filter the 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.

6 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 pink/white connective tissue remains. When finished, combine the tubes of cell suspension and proceed to the next section.

Ficoll-Paque

50m

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

8 Remove the supernatants and combine the cell pellets down to a single 50 mL tube, top to 50 mL with Room temperature DPBS-FBS-EDTA Solution.

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

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

11 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).

12 Remove the mononuclear cell layer from both tubes with a transfer pipet and combine in one 50 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.

13 Remove the supernatants and re-suspend the cell pellets in 50 mL cold DPBS-FBS-EDTA Solutio 10m and centrifuge the cell suspension for 00:10:00 at 120 x g, 4 °C.

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

Cell Count

15 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

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

17 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 30 million cells and discard/freeze/use any left over cells). Record the number of vials frozen: _____.