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Working

Adult Mouse Spleen Dissociation (On ice)

Version 3

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[1]CCHMC

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Human Cell Atlas Method Development Community

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ABSTRACT

Protocol used to dissociate adult (8-10 wk) mouse spleen into single cells. Attained >95% viability, a variety of cell sizes, and ~10 million cells from 12 mg tissue.

Spleen layered dissociation 2.20.19.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Collagenase Enzyme Mix (two tubes, 1 mL each)

7.5 mg/mL Collagenase A (Sigma, 10103578001)
7.5 mg/mL Collagenase Type 4 (Worthington, CLS-4)
100 µg/mL soybean trypsin inhibitor (Sigma, 10109886001)
125 U DNAse (Applichem, A3778)
5 mM $CaCl_2$
740 µL DPBS (no Ca, Mg)

+12 mg chopped spleen / tube

Preparing stock aliquots of reagents:

I make up 100 mg/mL stock of Collagenase A and Collagenase Type 4 (i.e. 100 mg dissolved in 1 mL of DPBS), aliquoted in 100 µL aliquots (10 mg per aliquot) and stored at -80 ºC.

For the soybean trypsin inhibitor, I make 1 mg/mL stock in DPBS and store 100 µg (100 µL) aliquots at -80 ºC.
The DNAse can be dissolved in DPBS, aliquoted and stored at -80 ºC.
For the Cacl2, I make up a 1 M stock and autoclave.

Required Equipment & Consumables:

Refrigerated centrifuge
Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters (MACS SmartStrainers, 130-098-458)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice (MLS)

Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Required reagents:
Red Blood Cell Lysis Buffer - Sigma (R7757)

The protocol workflow is as follows:
1. Isolate spleen
2. First layer
3. Second layer
4. Preparing cells for Chromium/DropSeq

MATERIALS

NAME	CATALOG #	VENDOR
Red Blood Cell Lysis Buffer Hybri-Max	R7757	Sigma Aldrich

BEFORE STARTING
-Set centrifuges to 4° C.
-Make two tubes of 1 mL enzyme mix.
-Make ~25 mL of DPBS/0.04% BSA (0.04% BSA/DPBS can be made by aliquoting 4 µL of 10% BSA stock to each mL of DPBS, i.e. 100 µL of 10% BSA stock for 25 mL DPBS).

Isolate Tissue

1 Isolate whole spleen and immerse/transport in ice-cold DPBS.

2 Using sterile forceps, transfer spleen to petri dish on ice. Remove excess DPBS using pipet. Chop whole spleen coarsely for 45 sec using razor blade on petri dish on ice until a fine paste. Manipulate tissue with forceps while mincing with razorblade to thoroughly break up.
00:00:45 mince on ice

First layer of dissociation

3 Weigh out 12 mg of minced spleen on petri dish. Using razor blade, transfer to 1.5 mL tube containing 1 mL of enzyme mix on ice.

4 Incubate tube on ice for 10 minutes. Triturate 10X every 2 mins and shake every min.
00:10:00 Incubate on ice **00:02:00 triturate 10X every 2 min** **00:01:00 Shake every min**

5 After 10 mins of digestion, let tissue chunks settle for 1 min on ice & save 80% of supernatant with released cells & apply cells to 30 µM filter on 50 mL conical, on ice. Rinse filter with 5 mL ice-cold PBS/0.04% BSA. Leave filter and 50 mL conical on ice, it will be used for the steps as well.
5 ml ice-cold PBS/BSA 0.04%

Second layer of dissociation

6 Add additional 1 mL enzyme mix to tissue chunks.

7 Continue to triturate 10x every 2 minutes and shake every minute while incubating on ice, for 10 additional minutes (21 min total time).
00:10:00 Incubate on ice **00:02:00 triturate 10x every 2 min** **00:01:00 shake every min**

8 After 21 min total time, triturate 10x and add entire volume of remaining digest mix to the same 30 µM filter on 50 mL conical as in the previous step. Rinse filter w/5 mL ice-cold PBS/0.04% BSA.

5 ml ice-cold PBS/BSA 0.04%

RBC Lysis

9 Transfer flow-through to 15 mL conical. Spin 650 g for five minutes at 4 °C. After spin, remove supernatant, leaving 100 µL volume in 15 mL conical tube.

4 °C **00:05:00 spin 650 g for 5 min**

10 Perform RBC lysis: add 1 mL RBC lysis buffer to the 100 µL of cells and triturate 10X. Let sit 3 minutes on ice. After incubating 3 min in RBC lysis buffer, add 13 mL ice-cold PBS/BSA 0.04% in the 15 mL conical to dilute the RBC lysis buffer. Pipet mix.

00:03:00 incubate on ice **13 ml ice-cold PBS/BSA 0.04%** **1 ml RBC lysis buffer**

Preparing cells for downstream analysis

11 Spin 650 g for 5 mins at 4 °C. Remove supernatant and re-suspend in 1 mL ice-cold PBS/BSA 0.04%.

4 °C **00:05:00 spin at 650 g for 5 min**

12 Examine cells using hemocytometer with trypan blue. Adjust concentration to 1000 cells / µL for 10x chromium or 100 cells / µL for DropSeq.