VERSION 2

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

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Adult Mouse Spleen Dissociation (On ice) V.2

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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.

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 CaCl₂
 740 µL DPBS (no Ca, Mg)

+12 mg chopped spleen / tube

MATERIALS

MATERIALS

Red Blood Cell Lysis Buffer Hybri-Max Sigma Aldrich Catalog
 #R7757

BEFORE START INSTRUCTIONS

- Set centrifuges to 4° C.
- Make two tubes of 1 mL enzyme mix.
- Make ~25 mL of DPBS/0.04% BSA

1 Isolate whole spleen and immerse in ice-cold PBS.

2 Using sterile forceps, transfer spleen to petri dish on ice. Chop whole spleen coarsely for 45 sec using razor blade on petri dish, on ice until a fine paste.

00:00:45 mince on ice

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 & remove 80% of supernatant with released cells & filter using 70 μ M 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%

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 cell digestion to 70 μ M filter on 50 mL conical, using the same filter and tube as in the previous step. Rinse filter w/5 mL ice-cold PBS/0.04% BSA.

5 mL ice-cold PBS/BSA
0.04%

- 9 Transfer flow-through to 15 mL conical. Spin 650 g for five minutes at 4 °C. After spin, remove supernatant, leaving 100 μ L 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 μ L of cells and triturate 10X. Let sit 3 minutes on ice. After incubating 3 min in RBC lysis buffer, add 10 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

10 mL ice-cold PBS/BSA
0.04%

1 mL RBC lysis
buffer

- 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 / μ L for 10x chromium or 100 cells / μ L for DropSeq.