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Adult Mouse Spleen Dissociation (On ice) V.2

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Human Cell Atlas Metho...

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

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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 $CaCl_2$
740 µL DPBS (no Ca, Mg)

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+12 mg chopped spleen / tube

Materials

MATERIALS

Red Blood Cell Lysis Buffer Hybri-Max **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7757

Before start

-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 containg 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 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 µL 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 µM 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 µL 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 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 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 µL ice-cold PBS/BSA 0.04%

1 µL 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 / µL for 10x chromium or 100 cells / µL for DropSeq.