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Adult Mouse Spleen Dissociation (On ice) V.1

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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)

+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 Chop tissue coarsely (~30 secs) using razor blade on petri dish, on ice.

2 Add 12 mg chopped tissue to 1 mL enzyme mix.

3 Incubate tube on ice for 10 minutes. Triturate 10X every 2 mins and shake every min.

00:10:00 Incubate on ice

4 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 Add additional 1 mL enzyme mix to tissue chunks.

6 Continue to triturate 10x every 2 minutes and shake every minute while incubating on ice, for 10 additional minutes.

00:10:00 Incubate on ice

7 Triturate and add entire volume of cell digestion to 70 µM filter on 50 mL conical. Rinse w/5 mL ice-cold PBS/0.04% BSA.

8 Transfer flow-through to 15 mL conical. Spin 650 g for five minutes at 4 °C. After spin, remove supernatant (down to ~100 µL).

9 Perform RBC lysis: add 1 mL RBC lysis buffer to cells and triturate 10X. Let sit 3 minutes on ice. Add 10 mL ice-cold PBS/BSA 0.04%

00:03:00 incubate on ice

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