Adult Mouse Spleen Dissociation (On ice)

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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.

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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

Before start

-Set centrifuges to 4° C.

-Make two tubes of 1 mL enzyme mix.

-Make ~25 mL of DPBS/0.04% BSA

Materials

Red Blood Cell Lysis Buffer Hybri-Max R7757 by Sigma Aldrich

Protocol

Step 1.

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

Step 2.

Add 12 mg chopped tissue to 1 mL enzyme mix.

Step 3.

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

Step 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.

Step 5.

Add additional 1 mL enzyme mix to tissue chunks.

Step 6.

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

Step 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.

Step 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).

Step 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%

Step 10.

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