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Version 1

Adult mouse lung cell 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

This protocol was used to dissociate adult (8-10 wk) mouse lung tissue. The entire procedure is carried out on ice (to reduce artifact gene expression changes) and takes about half an hour. The yield was 16,240 non-RBC/mg tissue with 87% viability.

Guidelines

Enzyme Mixes

Coll. A/Elastase/Dispase Enzyme Mix (1.5 mL)

90 µL Collagenase A 100 mg/mL – 6 mg/mL final (Sigma, 10103578001)

150 µL elastase 43 u/mL - 4.3 u/mL final (Worthington, LS002292)

150 µL Dispase 90 u/mL – 9 u/mL final (Worthington, LS02100)

7.5 µL 1 M CaCl₂ – 5 mM final

7.5 µL DNase (125 U/mL)

1095 µL PBS

→ save 0.5 mL of coll./elastase/dispase mix in separate 1.5 mL tube.

B. Lich enzyme mix (1 mL)

899 µL DPBS (no Ca, no Mg) - Thermo Fisher, 14190144

1 µL 0.5 M EDTA - 0.5 mM final conc.

100 µL *Bacillus Licheniformis* 100 mg/mL - 10 mg/mL final conc. - Sigma, P5380



Materials

MATERIALS

⊗ RBC Lysis Buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #R7757**

⊗ DNase **AppliChem Catalog #A3778**

⊗ BSA

Before start

- Prepare enzyme mixes and leave on ice.
- Cool centrifuges to 4 °C.

- 1 Mince lung tissue on petri dish on ice for 2 min until fine paste.
 00:02:00 mince tissue on ice
- 2 Weigh out 25 mg of tissue on petri dish. Using a sterile razor blade or forceps place 25 mg tissue in 1 mL 1enzyme mix in 1.5 mL eppendorf tube, incubating on ice.
 25 μ L minced lung tissue
- 3 Incubate on ice. Shake tube every 30 secs. Begin triturating at 2 mins. Triturate 10X every 1.5 minute (first w/tip cut).
 00:00:30 shake
 00:01:30 triturate 10x
- 4 After 5 min pipet tissue + enzyme mix into petri dish on ice. Mince 2 min using razor blade to further break up residual chunks of tissue.
 00:05:00 place digest mix in petri dish
 00:02:00 mince on petri dish using razor blade
- 5 Pipet tissue + enzyme back into 1.5 mL tube. Rinse petri dish with 0.5 mL coll. A/elastase/dispase enzyme mix and pipet into same tube.
 0.5 μ L rinse petri dish
- 6 Continue triturating on ice for 2 additional minutes until you reach 9 minutes total digestion time
 00:02:00 triturate on ice
- 7 At 9 min total digest time let tube settle for one min on ice. The chunks of tissue should settle to the bottom of the tube, leaving released cells in the supernatant. Pipet 80% of supernatant onto 70 μ M filter on sterile 50 mL conical.
 00:01:00 settle on ice
- 8 Rinse filter w/6 mL ice-cold PBS/BSA 0.04%. Leave filter on 50 mL conical for next steps.
 6 μ L ice-cold PBS/BSA 0.04%
- 9 Add additional 1 mL of 10 mg/mL b. lich enzyme mix to residual clumps of tissue in enzyme in the 1.5 mL tube.
 1 μ L B. lich enzyme mix
- 10 Continue triturating on ice 10x every 1.5 minute for 10 additional minutes (20 min total time). Shake every 30 sec.
 00:10:00 triturate on ice



00:01:30 triturate 10x

00:00:30 shake

- 11 Pipet entire volume to same 70 μ M filter - rinse w/7 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to 15 mL conical.

 7 μ L ice-cold PBS/BSA 0.04%

- 12 Spin 300 g for five minutes at 4 °C. Remove all but 100 μ L of supernatant - being careful not to disturb pellet.

4 °C spin at 300 g

- 13 Add 900 μ L RBC lysis buffer to pellet. Triturate 20X using 1 mL pipet set to 700 μ L and incubate for two min on ice.

 900 μ L RBC lysis buffer

00:02:00 incubate on ice

- 14 Add 12 mL ice-cold PBS/BSA 0.04% to 15 mL conical to dilute RBC lysis buffer.

 12 μ L ice-cold PBS/BSA 0.04%

- 15 Spin 15 mL conical 120 g for ten min at 4 °C to pellet cells and remove platelets (platelets should remain in the supernatant).

00:10:00 spin at 120 g

4 °C

- 16 Remove supernatant and re-suspend in 700 μ L ice-cold PBS/BSA 0.04%. Examine using hemocytometer w/trypan blue. Adjust concentration to 1000 cells / μ L for 10X Chromium or 100 cells / μ L for DropSeq.

 700 μ L ice-cold PBS-BSA 0.04%