Adult mouse lung cell dissociation (on ice) Version 2

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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.

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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

Required reagents:
Red Blood Cell Lysis Buffer - Sigma (R7757)

Optional Dead Cell Removal Kit:
EasySep dead cell removal (Annexin V) kit (cat. #17899)
EasySep Magnet (cat. #18000)

Required Equipment & Consumables:
Centrifuges for 1.5 mL and 15 mL conicals (MLS)
Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
70 µM filters - Miltenyi (130-098-462)
Petri dishes (MLS)

Razor blades (MLS)
Ice bucket w/ice (MLS)
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

The protocol workflow is as follows:
A. Isolate lung
B. First layer
C. Second layer
D. Preparing cells for Chromium

Before start

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

Materials

RBC Lysis Buffer R7757 by Sigma

DNAse A3778 by AppliChem

BSA by Contributed by users

Protocol

Step 1.
Mince lung tissue on petri dish on ice for 2 min until fine paste.

Step 2.
Weigh out 25 mg of tissue on petri dish. Using a sterile razor blade or forceps place 25 mg tissue in 1 mL enzyme mix in 1.5 mL eppendorf tube, incubating on ice.

AMOUNT

25 mg Additional info:
minced lung tissue

Step 3.
Incubate on ice. Shake tube every 30 secs. Begin triturating at 2 mins. Triturate 10X every 1.5 minute (first w/tip cut).

Step 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.

Step 5.
Pipet digest mix back into 1.5 mL tube. Rinse petri dish with 0.5 mL coll. A/elastase/dispase enzyme mix and pipet into same tube.

AMOUNT

0.5 ml Additional info:
rinse petri dish

Step 6.
Continue triturating on ice for 2 additional minutes until you reach 9 minutes total digestion time

Step 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 µM filter on sterile 50 mL conical.

Step 8.
Rinse filter w/6 mL ice-cold PBS/BSA 0.04%. Leave filter on 50 mL conical for next steps.

AMOUNT

6 ml Additional info: ice-
cold PBS/BSA 0.04%

Step 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.

AMOUNT

1 ml Additional info: B. lich
enzyme mix

Step 10.
Continue triturating on ice 10x every 1.5 minute for 10 additional minutes (20 min total time). Shake every 30 sec.

Step 11.
Pipet entire volume to same 70 µM filter - rinse w/6 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to 15 mL conical.

AMOUNT

6 ml Additional info: ice-
cold PBS/BSA 0.04%

Step 12.
Spin 300 g for five minutes at 4 ºC. Remove all but 100 µL of supernatant - being careful not to disturb pellet.

TEMPERATURE

4 °C Additional info: spin at 300 g

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

AMOUNT

900 µl Additional info: RBC lysis buffer

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

AMOUNT

12 ml Additional info: ice-cold PBS/BSA 0.04%

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

TEMPERATURE

4 °C Additional info:

Step 16.
Remove supernatant and re-suspend in 200 µL ice-cold PBS/BSA 0.04%.

AMOUNT

200 µl Additional info: ice-cold PBS-BSA 0.04%

Step 17.
Optional: to increase the % of viable cells, at this point in the procedure you can perform dead cell removal using the EasySep dead cell removal kit according to the manufacturer’s instructions.

Step 18.
Examine cells using hemocytometer w/trypan blue. Adjust concentration to 1000 cells / µL for 10X Chromium or 100 cells / µL for DropSeq.