Adult human lung cell dissociation (on ice)

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Abstract
This protocol was used to generate a single cell suspension from adult human lung tissue. The procedure is carried out on ice, reducing artifact gene expression changes.

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Guidelines

Enzyme Mixes

Coll. A/Elastase/Dispase Enzyme Mix (make two tubes - each 1 mL)
60 µL Collagenase A 100 mg/mL – 6 mg/mL final (Sigma, 10103578001)
100 µL elastase 43 u/mL - 4.3 u/mL final (Worthington, LS002292)
100 µL Dispase 90 u/mL – 9 u/mL final (Worthington, LS02100)
5 µL 1 M CaCl2 – 5 mM final
5 µL DNAse (125 U/mL)
730 µL DPBS (no Ca, no Mg)
+13 mg tissue per 1 mL enzyme mix

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

Protocol

Step 1.
Transport tissue in ice-cold PBS.

Step 2.
Mince tissue on petri dish on ice using razor blade for 2 min into 1-mm3 pieces.

Step 3.
Weigh out 13 mg tissue. Using a sterile razor blade or forceps place 13 mg tissue in 1 mL enzyme mix in 1.5 mL eppendorf tube, incubating on ice.

AMOUNT

13 mg Additional info:

minced human lung tissue

Step 4.
Incubate on ice. Triturate 10x using 1 mL pipet set to 700 µL every 3 min (w/tip cut). Shake 3-5X to re-suspend every 2 min.

Step 5.
After 45 minutes of incubation let settle on ice 1 min.

Step 6.
Remove 80% of the supernatant (consisting of released cells), leaving undigested tissue chunks on the bottom of the tube.

Step 7.
Add released cells to sterile 30 µM filter on 50 mL conical. Rinse filter w/15 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Step 8.
Divide flow-through into two 15 mL conicals. Bring the volume for each to 14 mL with ice-cold PBS/BSA 0.04%.

AMOUNT

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

Step 9.
Spin the two 15 mL conicals with released cells 650 g for 5 min at 4 °C.

TEMPERATURE

4 °C Additional info:

Step 10.
Remove supernatant for the 15 mL conicals with relesed cells. Re-suspend the pellets in 14 mL ice-cold PBS/BSA 0.04% for each tube.

AMOUNT

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

Step 11.
Add additional 1 mL enzyme mix to residual tissue chunks.

AMOUNT

1 ml Additional info: enzyme mix

Step 12.
Continue incubating on ice for 35 additional minutes (1 hr. 20 min. total). Triturate 10x using 1 mL pipet set to 700 µL every 5 min (w/tip cut). Shake 3-5X to re-suspend every 3 min.

Step 13.
After 1 hr. 20 min total incubation time triturate digest mix 10X and add digest mix to a new sterile 30 µM filter on 50 mL conical.

Step 14.
Rinse filter w/10 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to two 15 mL conicals.

AMOUNT

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

Step 15.
Bring the volume for each conical to 14 mL w/ice-cold PBS/BSA 0.04%.

Step 16.
Spin all four 15 mL conical tubes including the two from the previous step, 650 g for 5 min. at 4 ºC.

TEMPERATURE

4 °C Additional info:

Step 17.
Remove supernatant for all tubes. Re-suspend combined volume in 5 mL RBC lysis buffer. Pipet 20x to mix. Let incubate on ice 5 min.

AMOUNT

5 ml Additional info: RBC lysis buffer

Step 18.
Add 10 mL ice-cold PBS/BSA 0.04% to 5 mL RBC lysis buffer. Triturate and apply to sterile 30 µM filter on 50 mL conical.

AMOUNT

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

Step 19.
Transfer flow-through to two 15 mL conicals. Bring volume for each to 14 mL with ice-cold PBS/BSA 0.04%

AMOUNT

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

Step 20.
Spin 650 g for 5 min at 4 °C. Remove supernatant.

Step 21.
Re-suspend cells in 250 µL ice-cold PBS/BSA 0.04%. Analyze viability and cell yield using a hemocytometer with trypan blue.

AMOUNT

250 µl Additional info: ice-cold PBS/BSA 0.04%

Step 22.
Adjust cell concentration to 1000 cells / µL for 10x chromium or 100 cells/µL for DropSeq.