Jun 28, 2018 Version 1

Adult human lung cell dissociation (on ice) V.1

DOI
dx.doi.org/10.17504/protocols.io.rcad2se

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Human Cell Atlas Metho...

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Protocol Citation: Andrew Potter 2018. Adult human lung cell dissociation (on ice). **protocols.io** **https://dx.doi.org/10.17504/protocols.io.rcad2se**

Protocol status: Working
We use this protocol and it's working

Created: June 27, 2018

Last Modified: June 28, 2018

Protocol Integer ID: 13410

Keywords: lung, single cell, dissociation, CAP, adult human lung cell dissociation, adult human lung tissue, human lung tissue, single cell suspension, ice, artifact gene expression, reducing artifact gene expression change, artifact gene expression change

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.

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.

Troubleshooting

1 Transport tissue in ice-cold PBS.

2 Mince tissue on petri dish on ice using razor blade for 2 min into 1-mm3 pieces.
00:02:00 mince on ice

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.
13 mg minced human lung tissue

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.
00:03:00 triturate 10X
00:02:00 shake 3-5X

5 After 45 minutes of incubation let settle on ice 1 min.
00:45:00 incubate on ice
00:01:00 settle one min

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

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

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

9 Spin the two 15 mL conicals with released cells 650 g for 5 min at 4 °C.
00:05:00 spin at 650 g
4 °C

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.
14 mL ice-cold PBS/BSA 0.04%

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

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.

00:35:00 incubate on ice

00:05:00 triturate 10x

00:03:00 shake 3-5X

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.

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

10 mL ice-cold PBS/BSA 0.04%

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

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

00:05:00 spin 650 g

4 °C

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.

5 mL RBC lysis buffer

00:05:00 incubate on ice

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.

10 mL ice-cold PBS/BSA 0.04%

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

15 mL ice-cold PBS/BSA 0.04%

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

00:05:00 spin 650 g

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

250 µL ice-cold PBS/BSA 0.04%

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