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 We use this protocol and it's working

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

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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.

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.

- 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 μ L 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 μ M 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 released 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 μ L 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 μ M 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 °C.

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 μ M 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 μ L ice-cold PBS/BSA 0.04%. Analyze viability and cell yield using a hemocytometer with trypan blue.

🧪 250 μ L ice-cold PBS/BSA 0.04%

- 22** Adjust cell concentration to 1000 cells / μ L for 10x chromium or 100 cells/ μ L for DropSeq.