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Human Lung Digestion: Deriving a single cell suspension

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Human Lung Digestion: Deriving a single cell suspension

Digestion solution: (add PBS (without Ca,Mg) up to 50mL)

- 84mg Collagenase Type I (Life Tech, cat# 17100017, powder 285U/mg)

- 5mL Dispase (Fisher, cat# CB40235, 50U/mL)

- 3mL DNase1 (Roche, cat# 10104159001/165U/ml)

FACS Buffer:

- 1L dPBS (Cell Center, cat# 14190136, without Ca++ & Mg++)

- 2mL EDTA (Invitrogen, cat# 15575020, UltraPure, 0.5M, ph 8.0, 100mL)

- 10mL of 10%FBS (Invitrogen, cat# 10437-028, 500mL)

0.04% PBS+BSA (resuspending sample for sequencing)

- 10mg BSA (Jackson,cat# 001-000-162, IgG free, protease free)

- 25mL PBS (Cell Center, cat# 14190136, without Ca++ & Mg++)

MACS Accessories: (Miltenyi Biotec)

GentleMACS Octo Dissociator w/ heaters (cat# 130-096-427)

GentleMACS C Tubes (cat# 130-096-334)

MACS- Smart Strainers 70uM (4x25pk) (cat# 130-110-916)

PROTOCOL CITATION

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1 Prepare digestive solution and warm in 37C water bath until ready to use.

2 Make sure to turn on 4C centrifuge so it can get to temp.

3 Prep working space with bench paper and take out surgical tool box.

4 Make sure to prepare FACS Buffer and/or 0.04BSA+PBS, if needed, prior to starting experiment, keeping it on ice or in 4C.

5 Obtain ice bucket.

6 Retrieve sample from cold room and keep on ice.

7 Weigh the piece of lung, record any info on sample such as ID number, diagnoses, age, gender, and date.

8 Take histology samples for both 2% and 4% PFA fixation (2-3 small pieces about the size of your fingernail) and place in wheaton vials with PBS. Place vials on ice or in 4C until continuing with dehydration protocol for histology samples.

9 Carefully remove all pleura from sample (pleura will clog up digestion solution and filters).

10 10. Dissect out 4,000 mg of lung tissue, avoiding airways, by cutting small pieces and placing them on a separate petri dish on ice.

11. Using scissors, cut the dissected tissue into smaller pieces to improve digestion.

11

12 12. Collect 450-500 mg of dissected lung tissue into a gentleMACS tubes (for 4,000 mg, will have a total of 8 tubes).

13 13. Add 5-6ml of digestion solution to each tube. Less is more here as it helps the tissue sit next to the tines on the MACS tube cap. Be sure to close cap of gentleMACS tube tightly (this can be tricky, you want to feel it “pop” into place).

14 14. Place all gentleMACS tubes onto Dissociator. Snapping tubes into place, the screen will tell you the tube is on tightly.

15 15. Run the “m_lung_01_02” 2-3 times. This will do a gross breakup of the tissue. Check the MACS tubes after this – there should not be any large clumps of tissue in the fluid. Anything large should be stuck in the tines of the tube.

16 a. If there is an error, remove the cap and dislodge the tissue out of the cap into the bottom and repeat.

17 16. Add heating elements to tubes and run the “37C_m_LIDK_1” program for 35 minutes.

18 17. Run the “m_lung_01_02” program 1-2 more times to ensure total dissociation of tissue.

19 18. Filter samples through 70uM MACSsmart cell strainer into new 50mL tubes.

20 19. Wash gentleMACS tubes with 5mL PBS and filter.

21 20. Add 10mL PBS to each 70uM cell strainer. Pipetting up and down to let flow through. May need to remove large chunks of debris if clogging strainer up. Be careful not to pierce the strainer!

22 a. Note: You can combine two MACStubes into one falcon at this step, but may need to change the strainer for each sample to improve yield, but not always

23 b. You can also add an additional 10mL of PBS to strainer to increase cell yield

24 21. Spin at 350xg(rcf) for 5 min at 4C. (If pellet is too loose, spin for additional 3min)

22. Carefully discard supernatant into container with bleach solution.

23. Add 10mL of ACK Lysis buffer (Cell center, cat# 118-156-721EA) to lyse RBCs and shake gently to break up pellet.

24. Incubate at RT for 10 minutes.

25. Spin at 300xg(rcf) for 5 min at 4C. Discard supernatant.

a. If after the spin/wash, the pellet still has a lot of RBCs, you can repeat this step once with 5mL of lysis buffer, incubate for 5 minutes, spin for 5 minutes.

26. Wash pellet with 10mL FACS buffer and spin for 300g(rcf) for 5 min at 4C.

27. Discard supernatant and resuspend with 5mL FACS buffer.

28. Filter samples through 40uM cell strainers into 50mL conical tubes.

a. Two samples per 40uM strainer, so after this step should have total of 4 tubes

29. Wash the strainers with total of 10-15 mL of FACS buffer. This ensures we have all cells.

30. Spin at 300xg(rcf) for 5 min at 4C. Discard supernatant.

31. Resuspend with approximately 10-20mL of FACS (resuspension depends on pellet size).

a. If doing single cell sequencing resuspend in 0.04% PBS+BSA

32. Count cells on cellometer (Nexcelom, Cellometer K2), recording percent viability and total cell count.

39 33. Proceed to desired bead sorting protocol, cytospin protocol, single cell protocol, FACS staining, etc...