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URMC TriState SenNet Mouse Lung Digestion V.2

Version 1 is forked from Mouse Ischemia Experiment

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

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Disclaimer

The authors have no conflict of interest to declare.

Abstract

The objective of this document is to share the material and kits used and steps involved in digesting the mouse lungs to perform scRNA analyses. This study was performed for the TriState SenNet TMC Bioanalyses Core at the University of Rochester, as part of the Cellular Senescence Network Program (SenNet). This protocol ensures minimum damage to the cells in suspension and ensures maximum viability to conduct downstream analyses.

Materials

Materials, Equipment & Reagents including Formulations

1. Liberase: (Cat# 5401127001; Roche)
2. DNase I (Cat# 4380-096-06; R&D)
3. Serum free DMEM

4. Disposables:
i) C-tubes for GentleMACS (Cat# 130-093-237, Miltenyi Biotec)
ii) MACS SmartStrainers 70µm (Cat# 130-098-462, Miltenyi Biotec)
iii) Sterile serological pipets; 50, 25,10, 5, 2, 1 mL size
iv) Sterile pipet tips; 1000, 200 and 20 µl
v) Sterile 15mL or 50mL conical polypropylene tubes
vi) Test tube racks for 15mL or 50mL conical tubes
vii) LD columns (Cat# 130-042-901; Miltenyi Biotec)

5. Equipment:
i) GentleMACS Tissue Octo Dissociator (Miltenyi Biotec Inc San Diego, CA)
ii) MACSmix tube rotator
iii) Table Top Centrifuge
iv) Biological Safety Cabinet, Class II
v) Dissecting forceps and scalpel

Lung Tissue Dissociation

1 Mince tissue finely, place into 50ml GentleMACS C-tube with liberase enzymatic cocktail .

1.1 For 50mg tissue use 0.5 ml liberase + 2ml DMEM + 2ul 5 unit/ul DNase I.

2 Immediately transfer the tubes to MACS Tissue Dissociator and run user-defined program named m_lung_01_02 for mouse lungs.

3 Thereafter, place 50ml conical with digestion into an incubating rocker for 30 minutes at 37 °C .

NOTE: If you still see chunks of lung tissue in the tubes after this step then transfer to the MACS Tissue Dissociator and run user-defined program named m_lung_02_01 for mouse lungs for 10-15 sec.

4 Remove conical from rocker and strain the cell suspension through 70 micron into 50mL tube

5 Add 3 ml DMEM (10% FBS) through the strainer to collect the remaining cell suspension

6 After straining, centrifuge at 300g for 5 minutes at 4 °C .

7 Remove supernatant, add 500 µl of red blood cell lysis to cell pellet. Incubate the cell suspension On ice for 5 min.

8 After incubation add 4.5 mL of DMEM (10% FBS) to the suspension and centrifuge at 300g for 5 minutes at 4 °C .

9 Remove supernatant, re-suspend pellet in 2 mL DMEM (10%FBS)

10 Count cells and viability using AO/PI method, confirm reading under microscope