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WORKS FOR ME 1

UPitt TriState SenNet TMC Human lung single cell suspension (test run)

COMMENTS 0

DOI
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Marta Bueno[1], Lanping Guo[1], koenigshoffm[1], Oliver Eickelberg[1]

[1]Division of Pulmonary and Critical Care Medicine. Department of Medicine. School of Medicine

TriState SenNet

Cellular Senescence Network (SenNet) Method Development Community

Marta Bueno
Division of Pulmonary and Critical Care Medicine. Department...

ABSTRACT

Human lung tissue dissociation protocol to produce single cell suspension that can be used for different purposes. Optimal incubation times to obtain cell suspension with great cell numbers and high cell viability from non-disease lungs.

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Before you start

1 **Stock solutions**

Can be prepared beforehand & stored -20 °C:

1.1 Antibiotic-Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**:

Aliquot in 5 mL in sterile conditions.

Store at -20 °C

1.2 DNase I from bovine pancreas (Roche) **Sigma Aldrich Catalog #11284932001** 0 mg:

Stock of 100 mg in 10 mL of sterile ddH_2O

Aliquot in 300 µL in sterile conditions (3 mg per aliquot)

Store at -20 °C

1.3 Elastase (3U/mg) 100mg **Worthington Biochemical Corporation Catalog #LS002292**:

Stock of 100 mg in 1 mL of sterile ddH_2O

The enzyme should be 0.22 microns filtered after reconstitution and prior to use

Aliquot in 500 µL in sterile conditions (150U per aliquot)

Store at -20 °C

Collagenase IV (160U/mg) 1g **Worthington Biochemical Corporation Catalog #LS004188**:

Stock of 1 g in 10 mL of sterile ddH_2O

Aliquot in 500 µL in sterile conditions (8000U per aliquot)

Store at -20 °C

1.4 Liberase TL **Roche Catalog #05 401 020 001**:

Stock of 5 mg in 2 mL of sterile ddH_2O

Aliquot in 500 µL in sterile conditions (1.25mg per aliquot)

Store at -20 °C

2 **Same-day preparation:**

- DMEM high glucose HEPES **Thermo Scientific Catalog #12-430-062**
- RPMI-1640 **Thermo Fisher Scientific Catalog #22400089**
- FBS **Invitrogen - Thermo Fisher**
- 1X PBS (Phosphate-buffered saline)
- Red Blood Cell Lysis Buffer (Roche) **Sigma Aldrich Catalog #11814389001**

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2.1 Base media

500 mL DMEM + 5 mL anti/anti (100x)

2.2 Digestion media (10 mL per digestion -> scale accordingly)

I. Modified base media with Final DNAseI 100 µL per 10 mL (final 0.1mg/ml)

II. Then add corresponding proteases:

II.a. Elastase / Collagenase (EC tubes)

Final elastase 250 µL per 10 mL (final 7.5U/ml) +

Final collagenase IV 250 µL per 10 mL (final 400U/ml)

II.b Liberase TL (TL tubes)

Final Liberase TL 1 mL per 10 mL (final 0.25mg/ml)

2h

Tissue dissociation

3 Cut the lung tissue with a scalpel into approximately 3 cm^3 pieces (size of a thumb) upon receipt.

4 Place lung tissue into a 100mm petri dish. Place a ruler/control object next to it and take pictures for archives.

5 Transfer them to the lid of a fresh 100mm petri dish.

6 Each piece should be minced with scissors.
Locations: A-> Pleura; B-> bronchovascular bundle; C-> Parenchyma
(Label tubes accordingly)

A	B
EC-A	TL-A
EC-B	TL-B
EC-C	TL-C

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Table 1. Samples generated.

7 Transfer to a 50 ml conical Falcon tube with 10 mL added digestion media. Use one tube per piece of tissue.

8 Repeat the procedure with the remaining pieces.

9 Incubate **30min at 37C**, shaking 500rpm. 30m
500 rpm, 37°C, 00:30:00

10 Add 5 mL of FBS to the cell suspension to stop the digestion (final volume ~15ml)

11 Filter through 100 µm cell strainer using the syringe plunger to press and smash the tissue, washing the tube and rinse the strainer with 5 mL of plain RPMI.

12 Filter through 70 µm cell strainer using the syringe plunger to press and smash the tissue, washing tube and rinse the strainer with 5 mL of plain RPMI.

13 Centrifuge. 300g, 15°C for 10 minutes. 10m
300 x g, 15°C, 00:10:00

14 Remove and discard the supernatant.

15 Resuspend the cell pellets with 10 mL of plain RPMI and transfer to a fresh 15 ml Falcon conical tubes.

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15.1 Count cells here. (This count can be skipped -not informative because of RBC numbers)

15.2 To count: Take a 20 µL aliquot to determine total cell number, and mix with 20µl of VitaStain. Run AO/PI program in Nexcelom cell counter.

10m

16 Centrifuge. 300g, 15°C for 10 minutes.
300 x g, 15°C, 00:10:00

1m

17 Remove supernatant and resuspend the cell pellets with 5 mL of Red Blood Cell Lysis Buffer. Mix tubes by inversion and incubate for 00:01:00 at Room temperature.
(Note: It can be done by one after another)

18 Fill up the tubes with 9 mL of plain RPMI.

10m

19 Centrifuge. 300g, 15°C for 10 minutes.
300 x g, 15°C, 00:10:00

20 Remove and discard supernatant. Resuspend cell pellets with 10 mL of RPMI buffer kept on ice at all times.

20.1 Count cells here: Take a 20 µL aliquot to determine total cell number, and mix with 20µl of VitaStain. Run AO/PI program in Nexcelom cell counter.

10m

21 Centrifuge. 300g, 15°C for 10 minutes.
300 x g, 15°C, 00:10:00

22 Resuspend in the corresponding media for the next step

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22.1 For scRNA-seq:

- Resuspend in 500µl of PBS+2%FBS in the 15ml tube and then take 200µl of that suspension to a new Eppendorf tube.
- Pellet the cells by a centrifugation pulse, remove the supernatant and then resuspend to prepare ~4Million cells in 200µl of PBS+2%FBS (minimum 2M in 100µl)

22.2 For storage:

- Resuspend in 500µl of PBS
- Pellet the cells by a centrifugation pulse, remove the supernatant and stored at -80C

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