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 We use this protocol and it's working

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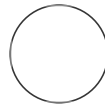
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# 🌐 MGH Harvard SenNet Processing murine lung for paired single cell RNA-seq and mass spec

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Cellular Senescence Network (SenNet) Method Development Community



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## ABSTRACT

Protocol for obtaining single cell suspension of murine lung.

## MATERIALS

PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free (Thermo Fisher Scientific; cat. no: AM9625)

1x PBS Buffer for washes and cell suspension

5ml Phosphate-Buffered Saline (10X) pH 7.4, RNase-free (Thermo Fisher Scientific; cat. no: AM9625).

45ml Invitrogen water

0.5% BSA in 1x PBS solution

Dissolve 0.250mg BSA in 1xPBS solution above.

DNase I (50,000x) 50,000 U/ml

Liberase Stock Solution (2.5mg/ml, 13 Wunsch units/ml)

To one 5mg vial, add 2ml cold Invitrogen ultrapure water. Mix on ice by periodically stirring. Can rock on rocking platform at 4°C for maximum 30min. Aliquot in 50-100ul aliquots (~40) and freeze at -20°C. Do not freeze thaw.

Enzyme master-mix

	Origin concentration	Volume for 4ml (per lung)
Liberase TM (Roche) (Cat# 05 401 119 001)	2,500ug/mL	160ul
DnaseI	50,000U/ml	5ul

RPMI 1640 (has Mg and Ca)		3.640 mL
FBS		200ul

RBC Lysis buffer (commercial, Thermo Fisher Scientific, 00-4333-57)f

100% FBS

DMSO

## Dissection

- 1 Euthanize mice by CO2 method.
- 2 Perfuse lungs via right ventricle.
- 3 Dissect out trachea and lungs and place in HBSS on ice.

## Single cell suspension

- 4

- 5 Pipet 1ml digestion solution on the lid of a 10cm dish. Place the lung in it. Using a razor blade, "mince" the lung, holding one tip with the forceps.
- 6 Pipet the sample into 4ml of the digestion solution / lung (8ml per mouse) and incubate at 37°C for 30min with rocking (in a 15ml falcon tube).
- 7 Use a Pasteur pipet to triturate suspension until tissue is fully dissociated.
- 8 Pass the suspension through a 100  $\mu$ m cell strainer into 50-mL tubes and use 10 mL of DPBS to pass through the strainer to wash it.
- 9 Centrifuge at 1000  $\times$  g for 5 min at 4°C, discard the supernatant.
- 10 Add 1-2 mL of 1 $\times$  RBC lysis buffer to resuspend the pellet and incubate for 5 min at 20°C–26°C.
- 11 Add 10 mL of DPBS to stop the lysing,
- 12 Centrifuge at 1000  $\times$  g for 5 min at 4°C, discard the supernatant.

- 13 Resuspend with 200  $\mu$ L of 0.5% BSA in PBS. Keep on ice.

## Count cells with heamocytometer

- 14 Count two samples each per lung and note down the exact number of cells. This information is important for scRNA library prep. We routinely recover 8-10 million cells / mouse with this protocol.

## Prepare samples for scRNA-seq and scMS

- 15 Split lung samples into 100,000 cell aliquots, according to the cell counts determined above.
- 16 Keep one aliquot on ice for the scRNA-seq sample.
- 17 For the remaining aliquots, spin down at  $1000 \times g$  for 5 min, discard the supernatant and resuspend in 90% FBS, 10% DMSO and freeze at  $-80^{\circ}\text{C}$ . These are the scMS samples.

## scRNA library preparation

- 18 Prepare according to manufacturer's protocol:

 CG000204\_ChromiumNextGEMSingleCell3\_v3.1\_Rev\_D.pdf