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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 Net...

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

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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
5mlPhosphate-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

Troubleshooting

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 µm cell strainer into 50-mL tubes and use 10 mL of DPBS to pass through the strainer to wash it.

9 Centrifuge at 1000 × g for 5 min at 4°C, discard the supernatant.

10 Add 1-2 mL of 1× 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 × g for 5 min at 4°C, discard the supernatant.

13 Resuspend with 200 uL 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 × g for 5 min, discard the supernatant and resuspend in 90% FBS, 10% DMSO and freeze at -80∗C. These are the scMS samples.

scRNA library preparation

18 Prepare according to manufacturer's protocol:

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Protocol references

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