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Single Cell Dissociation of Fresh Lung Tissue

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1 Works for me dx.doi.org/10.17504/protocols.io.7xkhpkw

Human Cell Atlas Method Development Community

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TGen

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/753806v1

GUIDELINES

All practices follow all safety guidelines regarding human tissue handling.

MATERIALS

NAME	CATALOG #	VENDOR
Collagenase Type 1	LS004197	Worthington Biochemical Corporation
Collagenase Type 4	LS004188	Worthington Biochemical Corporation
Neutral Protease (Dispase)	LS02106	Worthington Biochemical Corporation
DNase I RNAse & Protease-free	LS006331	Worthington Biochemical Corporation
HBSS	14025-092	Gibco - Thermo Fischer
PBS without Ca2 and Mg2	21-040-CV	Corning
100um cell strainer	10199-658	VWR International
MACS SmartStrainers 30um	130-098-458	Miltenyi Biotec
GentleMACS C tube	130-093-237	Miltenyi Biotec
Bovine Albumin Fraction V (7.5% solution)	15260037	Gibco, ThermoFisher

Lung Dissociation

1 Transfer distal lung tissue to a cold petri dish and dissect into required pieces for the experiment

2 One piece tissue (2cm^3) blot dry on disposable underpad and place to another cold petri dish. Mince the tissue into smaller pieces using scalpel. Transfer minced tissue to a GentleMACS C tube containing **7 ml** of **4 °C** Enzyme Mix.

Reagent	Catalog Number	Final Concentration
Collagenase Type I	LS004197	1 mg/ml
Collagenase Type 4	LS004188	1 mg/ml
Neutral Protease (Dispase)	LS02106	1 mg/ml
DNase I (RNAse & Protease-free)	LS006331	10 ug/ml
HBSS	14025-092	to 7 ml

Enzyme Mix

3 Place on GentleMACS Dissociator using the program **GM8.zip** for **00:18:00** at **37 °C** .

Program installation on GentleMACS:
1. Unzip GM8.zip and place GM8 folder in a **blank** USB.
2. With machine on, insert USB
3. Select the Editor menu -> USB -> Select the program in GM8 folder -> "Save" and select desired destination folder

4 Add **7 ml** **4 °C** Inactivation Media and use a 10 ml serological pipette to titrate 5-10 times 2 ml/s. Using same pipette, pass the cell suspension through series 100 µm, 30 µm strainer into a 15 ml falcon tube.

Reagent	Catalog Number	Final Concentration
FBS	16000044	10%
PBS	21-040-CV	1X

Inactivation Media

5 Centrifuge 300g for **00:05:00** at **4 °C** .

6 Remove the supernatant. Resuspend the cell pellet in **5 ml** **4 °C** 1X PBS 3% BSA.

Reagent	Catalog Number	Final Concentration
BSA (7.5%)	15260037	3%
PBS	21-040-CV	1X

1X PBS 3% BSA

7 Count cell numbers. Transfer 1 - 2 x 10^6 cells to a new 15 ml falcon tube.

8 Centrifuge 300g for **00:05:00** at **4 °C** . Resuspend the cell pellet in **1 ml** **4 °C** 1X PBS 3% BSA.

9 Add **2 µl** of 50 µM calcein AM to the cell suspension.

10 Incubate for **00:15:00** to **00:20:00** at **Room temperature** , protected from light.

Single Cell 5' Library

11 Follow 10X Genomics Chromium Single Cell 5' Library Kit. Prepare cell collection tubes containing RT Reagent Mix, Poly-dT RT Primer, Additive A, and nuclease-free water.

CG000086_ChromiumSingleCellV_D_J_ReagentKits_UG_RevH.pdf

Reagent	Product Number	1X (µl)
RT Reagent Mix	220089	50
Poly-dT RT Primer	2000007	5.9
Additive A	220074	2.4
Nuclease-free Water	NA	16.7
Single cell suspension	NA	~15 - 16 (see next step)
RT Enzyme Mix B	2000010 or 2000021	10 (added last)
Total		100

12 Immediately after incubation, FACS (Sony SH800, 70 µm sorting chip) sorting 15,000 calcein AM positive cells into collection tubes at **4 °C** .

15,000 cells is about 15 - 16 µl using Sony SH800 with a 70 µm sorting chip.

We recovered 3,000 - 4,000 cells post sequencing from loading 15,000 cells. Number of cells for loading depends on targeted cell recovery and your sample recovery efficiency. Variable factors include: sorting method, time, and nozzle diameter, 10x Chromium loading efficiency, and individual sample quality. ie. Sorting 15,000 cells (70 µm nozzle) directly to the RT buffer (minus RT Enzyme Mix B) required an additional 16.7 µl nuclease-free water to reach 100 µl total reaction volume, where using a 100 µm nozzle would require less nuclease-free water.

13 Add 10 µl of RT Enzyme Mix B to the reaction mix + cell suspension for a total of 100 µl.

14 Load Chromium chip A and run Chromium controller for GEM generation.

15 Generate cDNA and library following the kit protocol.