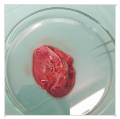

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Working

Cell dissociation of fresh human lung tissue for single-cell RNA-seq

Ilias Angelidis[1], Maximilian Strunz[1], Herbert Schiller[1]

[1]Helmholtz Zentrum Munich

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Human Cell Atlas Method Development Community

Ilias Angelidis

EXTERNAL LINK

https://www.helmholtz-muenchen.de/ilbd/research/ilbdcpc-junior-research-groups/systems-medicine-of-chronic-lung-disease-schiller-lab/scientific-focus/index.html

GUIDELINES

Human body fluids and tissue potentially contain blood borne viruses and other agents. Work with blood samples or tissue from individuals therefore carries a risk of infection if the material is not handled with care.

All practices must follow all safety guidelines regarding human tissue handling.

MATERIALS

NAME	CATALOG #	VENDOR
DNAse I, RNAse-free	79254	Qiagen
PBS without Ca2 or Mg2	10010-031	Gibco, ThermoFisher
Dispase	354235	Corning
Collagenase CLS I	C1-28	Biochrom AG
Elastase	20931	Serva, Germany
FBS	S 0615	Biochrom AG

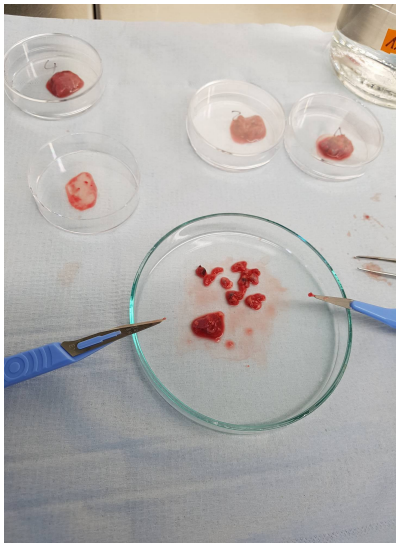
STEPS MATERIALS

NAME	CATALOG #	VENDOR
RBC Lysis Buffer	00-4333-57	Invitrogen - Thermo Fisher

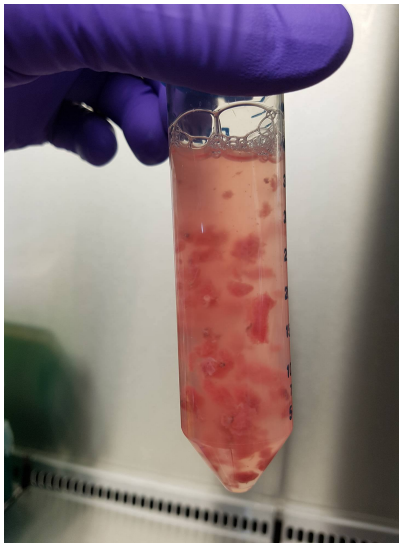
1 Transfer lung tissue in a petri dish and cut the required amount for your experiment (2x2x1)cm.

Desired amount of tissue is cut off

2 Mince the tissue into small pieces using a pair of scissors. Transfer tissue chunks in a 50mL Falcon tube containing **30 ml** of ice cold PBS. This step is intended to wash away any remaining blood off the tissue.



Tissue is cut into small pieces



Tissue is washed in PBS

3 Remove PBS by passing the minced tissue through a 40µm strainer and keeping the tissue pieces on top.

Tissue is now minced, washed and ready for Digestion.

4 Transfer the tissue into a new 50mL Falcon tube containing **8 ml** of **Enzyme Mix** (should suffice for 2x2x1 cm of tissue).

Enzyme	Cat. Number	Final Concentration
Dispase	354235 (Corning)	50 caseinolytic units/ml
Collagenase	C1-28 (Merck)	0,6 mg/ml
Elastase	20931 (Serva)	0,02 mg/ml
DNase	79254 (QIAGEN)	17 units/ml

Enzyme Mix

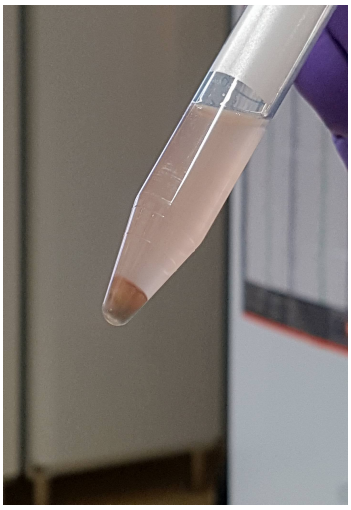
5 Incubate for: **00:50:00** at **37 °C** with constant shaking at 750rpm.

6 After enzymatic digestion add **7 ml** of ice cold **Inactivation Buffer.** Mix well using a 10mL serological pipette and pass the cell suspension from the digested tissue through a 70µm strainer into a 15mL Falcon tube (keep flow-through).

Reagent	Cat. Number	Final Concentration
PBS	10010-015 (gibco)	1X
FBS	S 0615 (Merck)	10%

Inactivation Buffer

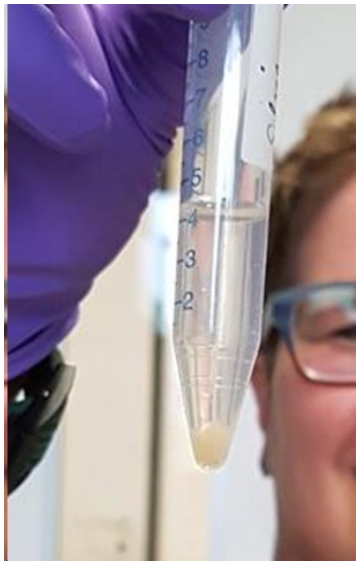
7 Centrifuge the cell isolate at **300g** for **00:05:00** at **4 °C** .



Typical cell pellet

8 Resuspend cell pellet in **2 ml** of **RBC Lysis Buffer** at **RT** for **00:02:00** to remove remaining red blood cells.

RBC Lysis Buffer
by Invitrogen - Thermo Fisher
Catalog #: 00-4333-57



Cell pellet after RBC

9 Add **10 ml** of ice cold **Inactivation Buffer** to inhibit the activity of the RBC Lysis Buffer.

10 Centrifuge the cell isolate at **300g** for **00:05:00** at **4 °C** .

11 Resuspend in **1 ml** of ice cold **Inactivation Buffer** and count the cells.

12 Assess cell viability using Trypan Blue staining. (cell viablity must be around 85%-95% in order to yield high quality single cell libraries)

13 Proceed with preferred scRNA-seq platform using the appropriate number of cells.