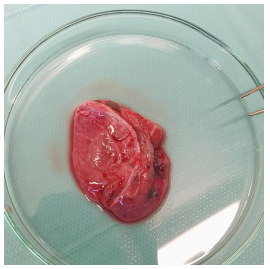
Apr 18, 2019

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



DOI
https://dx.doi.org/10.17504/protocols.io.zp2f5qe

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Human Cell Atlas Metho...

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Protocol Citation: Ilias Angelidis, Maximilian Strunz, Herbert Schiller 2019. Cell dissociation of fresh human lung tissue for single-cell RNA-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.zp2f5qe

Manuscript citation:

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

Created: April 02, 2019

Last Modified: April 18, 2019

Protocol Integer ID: 21978

Keywords: Single cell, Lung Tissue, scRNAseq, cell dissociation of fresh human lung tissue, single cell rna, cell rna, cell dissociation, single cell, fresh human lung tissue, rna, seq

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

MATERIALS

- DNAse I, RNAse-free **Qiagen Catalog #**79254
- PBS without Ca2 or Mg2 **Gibco - Thermo Fisher Scientific Catalog #**10010-031
- Dispase **Corning Catalog #**354235
- Collagenase CLS I **Biochrom AG Catalog #**C1-28
- Elastase **Serva, Germany Catalog #**20931
- FBS **Biochrom AG Catalog #**S 0615

STEP MATERIALS

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

Protocol materials

- DNAse I, RNAse-free **Qiagen** **Catalog #**79254
- PBS without Ca2 or Mg2 **Gibco - Thermo Fisher Scientific** **Catalog #**10010-031
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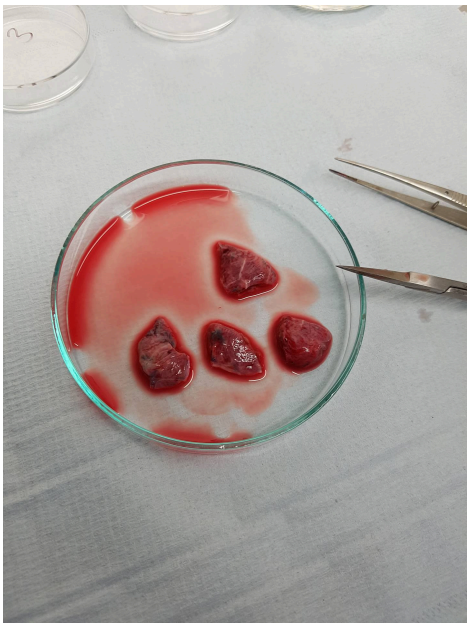
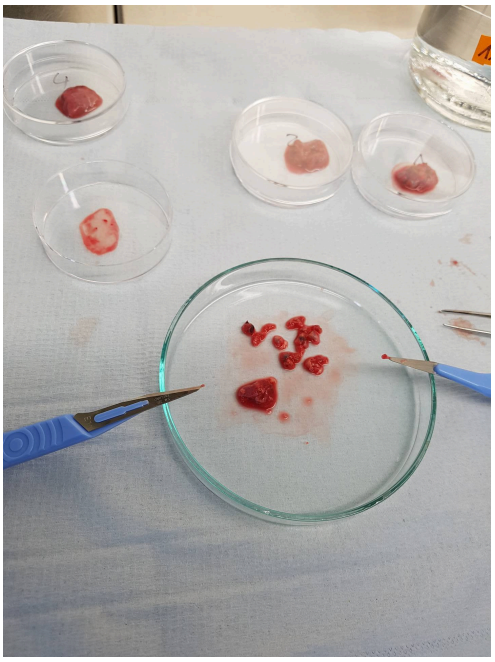
- FBS **Biochrom AG** **Catalog #**S 0615
- RBC Lysis Buffer **Invitrogen - Thermo Fisher** **Catalog #**00-4333-57

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

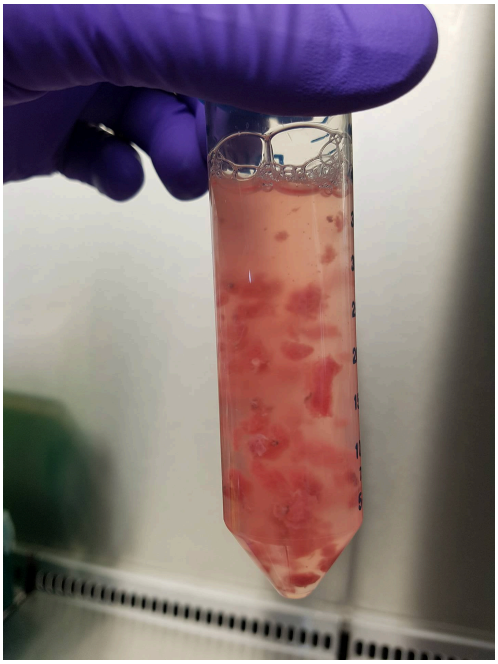


Desired amount of tissue is cut off

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 2×2×1 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

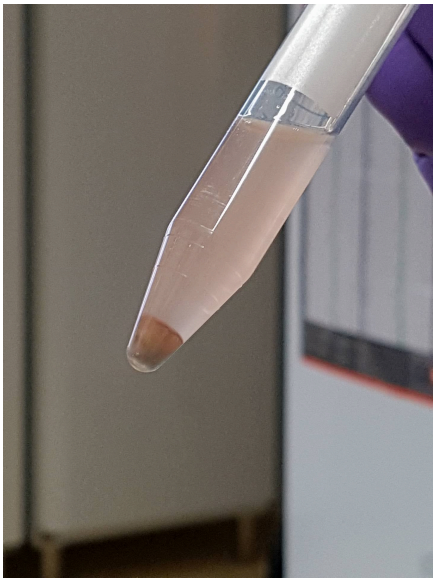
5 Incubate for: 00:50:00 at 37 °C with constant shaking at 750rpm.

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

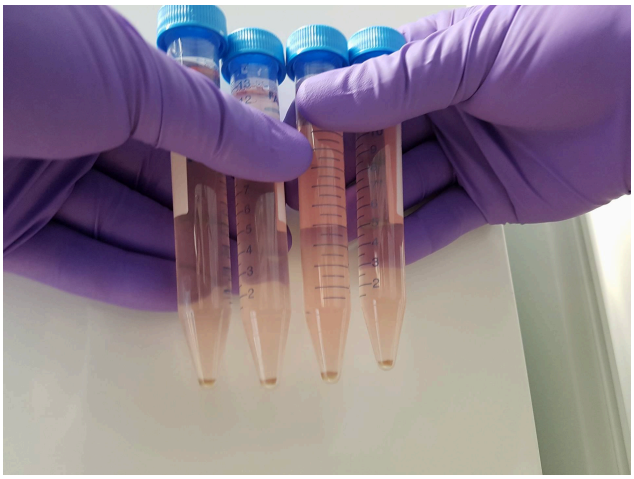
7 Centrifuge the cell isolate at **300g** for 00:05:00 at 4 °C .



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 **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.