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

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Human Cell Atlas Method Development Community

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

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- DNAse I, RNAse-free Qiagen Catalog #79254
- PBS without Ca²⁺ or Mg²⁺ Gibco, ThermoFisher 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

MANUSCRIPT CITATION:

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

Desired amount of tissue is cut off

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 $8 \text{ mL}$ of Enzyme Mix (should suffice for 2x2x1 cm of tissue).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cat. Number</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispa 5e</td>
<td>354235 (Corning)</td>
<td>50 caseinolytic units/ml</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Collagenase</strong></td>
<td>C1-28 (Merc k)</td>
<td>0.6 mg/ml</td>
</tr>
<tr>
<td><strong>Elastase</strong></td>
<td>20931 (Serv a)</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td><strong>DNase</strong></td>
<td>79254 (QIAG EN)</td>
<td>17 units/ml</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Cat. Number</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>10010-015 (gibco)</td>
<td>1X</td>
</tr>
<tr>
<td>FBS</td>
<td>S0615 (Merc k)</td>
<td>10%</td>
</tr>
</tbody>
</table>

**Inactivation Buffer**

7. Centrifuge the cell isolate at 300g for 00:05:00 at 4 °C.
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
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 viability must be around 85%-95% in order to yield high quality single cell libraries)
Proceed with preferred scRNA-seq platform using the appropriate number of cells.