Cell dissociation from airway biopsies with cold-active protease for single-cell RNA-seq

Laure-Emmanuelle Zaragosi1, Pascal Barbry1

1Université Côte d'Azur, CNRS, IPMC, 06560 Valbonne, France

Human Cell Atlas Method Development Community

ABSTRACT

This protocol provides details on the cell dissociation that should be performed to obtain single-cell suspensions from airway biopsies. Biopsies may come from tracheal, bronchial or nasal epithelium. Cell dissociation is performed at 4°C to avoid gene expression alterations and maximize viability. The typical cell number recovery is 40 000 cells for one biopsy. Cell suspensions are suitable for single-cell RNA-sequencing protocols.

GUIDELINES

Storage Conditions of Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>4°C</td>
</tr>
<tr>
<td>20 mM EDTA</td>
<td>room temperature</td>
</tr>
<tr>
<td>BSA (Sigma, A8806)</td>
<td>4°C</td>
</tr>
</tbody>
</table>

Protocol Citation: Laure-Emmanuelle Zaragosi, Pascal Barbry 2019. Cell dissociation from airway biopsies with cold-active protease for single-cell RNA-seq. protocols.io https://dx.doi.org/10.17504/protocols.io.x3efqje

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Protocol status: Working

We use this protocol and it's working

Created: Feb 12, 2019
Last Modified: Feb 13, 2019

PROTOCOL integer ID: 20294

Keywords: bronchial biopsy, airway epithelium, single-cell, dissociation, cold-active protease

<table>
<thead>
<tr>
<th>Proteinase from <em>Bacillus Licheniformis</em> (Sigma, P5380)</th>
<th>Store 100 μL aliquots (100 mg/mL) in DPBS at -80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoest 33342 (10 mg/mL)</td>
<td>4°C</td>
</tr>
<tr>
<td>NucGreen™ Dead 488 Ready Probes™ Reagent</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

Required Equipment

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Countess FL automated cell counter</td>
<td>ThermoFisher Scientific</td>
<td>AMQ AF1000</td>
</tr>
</tbody>
</table>

The protocol workflow is as follows:

1. Perform airway biopsies in the desired zone
2. Dissociation: mince with scalpel then triturate on ice in dissociation buffer
3. Remove red blood cells if necessary
4. Prepare cells for Chromium/DropSeq

*All steps should be performed on ice or at 4°C*
Use wide-bore 1 mL pipet tip for all biopsy transfers.

## MATERIALS

- EDTA Contributions by users
- 23G Needles Contributions by users Catalog #4657667
- Protease from Bacillus Licheniformis Sigma Catalog #P5380
- Quick-Read 10 Chamber Slide Globe Scientific Catalog #3805
- Countess™ Cell Counting Chamber Slides Contributions by users Catalog #C10314
- DPBS no calcium, no magnesium Invitrogen - Thermo Fisher Catalog #14190136
- 21G needle VWR international Ltd Catalog #BD-305165
- HBSS Gibco - Thermo Fischer Catalog #14060040

## STEP MATERIALS

- Quick-Read 10 Chamber Slide Globe Scientific Catalog #3805
- Ammonium Chloride Solution 100 mL Stemcell Technologies Catalog #7800
- Flowmi cell strainer Contributions by users Catalog #H13680-0040
- Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water Thermo Fisher Scientific Catalog #H3570
- NucGreen™ Dead 488 ReadyProbes™ Reagent Thermo Fisher Scientific Catalog #R37109
<table>
<thead>
<tr>
<th><strong>PROTOCOL MATERIALS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water Thermo Fisher Scientific Catalog #H3570</td>
</tr>
<tr>
<td>Materials, Step 25</td>
</tr>
<tr>
<td>DPBS no calcium, no magnesium Invitrogen - Thermo Fisher Catalog #14190136</td>
</tr>
<tr>
<td>DPBS no calcium, no magnesium Invitrogen - Thermo Fisher Catalog #14190136</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>Quick-Read 10 Chamber Slide Globe Globe Scientific Catalog #3805</td>
</tr>
<tr>
<td>Materials, Step 25</td>
</tr>
<tr>
<td>Quick-Read 10 Chamber Slide Globe Globe Scientific Catalog #3805</td>
</tr>
<tr>
<td>Countess™ Cell Counting Chamber Slides Contributed by users Catalog #C10314</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>23G Needles Contributed by users Catalog #4657667</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>HBSS Gibco - Thermo Fischer Catalog #14060040</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>EDTA Contributed by users</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>Ammonium Chloride Solution 100 mL STEMCELL Technologies Inc. Catalog #7800</td>
</tr>
<tr>
<td>Materials, Step 11</td>
</tr>
<tr>
<td>Flowmi cell strainer Contributed by users Catalog #H13680-0040</td>
</tr>
<tr>
<td>Materials, Step 21</td>
</tr>
<tr>
<td>Protease from Bacillus Licheniformis Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5380</td>
</tr>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>21G needle VWR International Catalog #BD-305165</td>
</tr>
</tbody>
</table>

**SAFETY WARNINGS**

Samples coming from patients with undetermined viral status should be process in cell culture rooms with the appropriate safety level.
BEFORE START INSTRUCTIONS

Prepare *Bacillus Licheniformis* enzyme mix just prior to starting dissociation:

<table>
<thead>
<tr>
<th>Volume (µl)</th>
<th>Reagent</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>850</td>
<td>DPBS</td>
<td>1X</td>
</tr>
<tr>
<td>50</td>
<td>20 mM EDTA</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>100</td>
<td>Protease from <em>B. Licheniformis</em> (100 mg/mL)</td>
<td>10 mg/mL</td>
</tr>
</tbody>
</table>

Prepare Inactivation buffer:

Make stock of 10% BSA in HBSS and store at -20 °C. To make HBSS/BSA 2% aliquot 40 mL of HBSS in 50 mL conical and pipet in 10 mL of 10% BSA stock.

Prepare Wash buffer:

To make HBSS/BSA 1% aliquot 20 mL of HBSS in 50 mL conical and pipet in 20 mL of HBSS/BSA 2%.

Prepare Resuspension buffer:

To make HBSS/BSA 0.05% aliquot 1 mL of HBSS/BSA 2% in 50 mL conical and pipet in 39 mL of HBSS.

Prepare cell staining reagent:

- HBSS: 500 µL
- Hoechst 33342 (10 mg/mL): 1 µL
- NucGreen™ Dead 488 ReadyProbes™ Reagent: 1 drop

1 Perform bronchial biopsy at the desired level of the airways (to be performed by a medical doctor)
Equipment

<table>
<thead>
<tr>
<th>Biopsy forceps</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medi-Globe</td>
<td>BRAN D</td>
</tr>
<tr>
<td>GBF-21-18-120</td>
<td>SKU</td>
</tr>
</tbody>
</table>

2. Put the biopsy in 1 mL DPBS in a well of a 6-well plate, observe aspect, and then transfer into 1 mL of ice-cold dissociation buffer in a 1.5 mL eppendorf tube. Use wide-bore 1 mL pipet tip for all biopsy transfers.

Note

PREPARATION OF DISSOCIATION MIX (Fresh at each experiment)

Ingredients:
- PBS
- Protease from Bacillus Licheniformis (100 mg/mL stock solution in PBS)
- EDTA 10 mM

For 1 mL of dissociation mix add:
- 850 microlitres of PBS
- 100 microlitres of protease (Final concentration: 10 mg/mL)
- 50 microlitres of EDTA (Final concentration: 0.5 mM)
3 If transportation or storage is necessary: place tube on ice or in polystyrene box containing ice packs. Biopsies can be stored for 60 min in dissociation buffer.

4 Carefully remove biopsy from the dissociation buffer, with a wide-bore 1 mL pipet tip and place in a 100 mm petri dish, taking as little dissociation buffer as possible. Mince with a scalpel equipped of a 10 blade. Drag the biopsy out of the liquid and mince very carefully into the smallest possible pieces. With a wide-bore pipet tip, transfer back the minced biopsy with a small
volume of dissociation buffer. Rinse the petri dish with dissociation buffer, at the location of biopsy mincing to recover as many cells as possible.

5 Incubate cells on ice for 90 to 120 min after mincing, with gentle trituration with needles 5 times every 5 min. Use needle with decreasing sizes from 21G to 23G.

6 Inactivate protease by adding 200 µL of Inactivation buffer (HBSS containing 2% BSA)

*Note*

**Prepare Inactivation buffer:**

- HBSS : 40 mL
- 10% BSA stock: 10 mL

7 Spin at 400g for 5 min at 4°C

8 Discard supernatant leaving 10 µL of residual liquid on the pellet.

9 Resuspend in 100 µL of wash buffer (HBSS + 1% BSA)

*Note*

**Prepare Wash buffer:**

- HBSS : 20 mL
- HBSS/BSA 2%: 20 mL
10 Observe cells under an inverted microscope to evaluate red blood cells (RBC) content. RBC content is better evaluated using an automated cell counter such as Countess, after addition of Hoechst 33342 to an aliquot of the cell suspension to discriminate nucleated cells from non-nucleated cells.

11 If RBC content is lower than 50%, go directly to step 18.
Perform RBC lysis: add 900 µL of Ammonium Chloride 0.8% to 100 µL of cell suspension (9 volumes).

Ammonium Chloride Solution 100 mL
Stemcell Technologies Catalog #7800
900 µL Ammonium Chloride 9 vol. for 1 cell vol.

12 Incubate on ice for 10 min.

00:10:00 RBC lysis
4 ºC

13 Add 200 µL of Inactivation buffer

200 µL Inactivation buffer

14 Spin at 400g for 5 min at 4°C

15 Discard supernatant leaving 10 µL of residual liquid on the pellet.

16 Resuspend in 100 µL of wash buffer and monitor correct RBC lysis under microscope

100 µL wash buffer

17 Add 1 mL of wash buffer

1 mL wash buffer
18 Spin at 400g for 5 min at 4°C

19 Discard supernatant leaving 10 µL of residual liquid on the pellet.

20 Resuspend in 500 µL of wash buffer

21 Filter cell suspension through Flowmi cell strainer

22 Add 500 µL of wash buffer to filtered cells.

23 Discard supernatant leaving 10 µL of residual liquid on the pellet.

24 Resuspend in 40 µL of resuspension buffer (HBSS + 0.05% BSA).

---

**Note**

**Prepare Resuspension buffer:**

- HBSS : 39 mL
- HBSS/BSA 2%: 1 mL
Mix 10 µL of cells with 10 µL of cell counting solution (HBSS with 20 µg/mL Hoechst 33342 and NucGreen). Incubate for 1 min at room temperature.

**Note**

**Preparation of cell staining reagent:**

- HBSS: 500 µL
- Hoechst 33342 (10 mg/mL): 1 µL
- NucGreen™ Dead 488 ReadyProbes™ Reagent: 1 drop

Count with Countess automated cell counter using both sides of chambers. Monitor the percentage of nucleated cells (Hoechst +) and dead cells (GFP+).
Countess GFP image after NucGreen and Hoescht33342 staining
Countess Dapi image after NucGreen and Hoescht33342 staining
Countess report after NucGreen and Hoescht33342 staining
Adjust concentration to a range of 700 to 1000 cells/µL (with wash buffer) for 10X Chromium. Monitor final cell concentration.

Floid image after NucGreen and Hoescht33342 staining