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Single-cell suspension preparation from Human bronchial biopsies to perform scRNA-sequencing using 10x chromium

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol enables the dissociation of human bronchial biopsies into a single-cell suspension starting from fresh materials. These single cells are suitable for use in 10x chromium kits for single-cell RNA sequencing.

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KEYWORDS

Human bronchial biopsies, Single-cell suspension, 10x chromium, scRNA sequencing, discovAIR

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MATERIALS TEXT

Solution/buffers required:

1. Tissue dissociation solution per sample

Reagents	Required amount
*HBSS +1% P/S	3ml
DNase I (Roche Diagnostics GmbH, REF #: 10104159001)	0,0003 grams
Collagenase D (Roche Diagnostics GmbH, REF #: 11088866001)	0,003 grams

*Hanks' Balanced Salt solution (HBSS with calcium, magnesium and phenol red, 500mL, Lonza, Cat #: BE10-508F), supplemented with 1% P/S (Penicillin-Streptomycin, Gibco, Cat #:150700630).

2. Resuspension buffer

Reagents	Required amount
PBS pH 7.4 (Gibco, REF #:10010-15, LoT #: 2288905)	1x
BSA (7.5% in DPBS, SIGMA, Cat #: A8412)	0.04% (400µg/ml)

To prepare resuspension buffer in 50mL volume, take (266.66µl) from the 7.5% BSA stock solution and fill the tube up to 50mL with 1x PBS solution.

3. Red blood cells lysis buffer (Ammonium Chloride Kalium, ACK 10x), pH 7.4

Reagents (conc)	Required amount
155mM Ammoniumchloride (NH4Cl) (Sigma-Aldrich, CAS #: A9434)	41.45 grams
10mM Kaliumhydrogencarbonat (KHCO3) (Merck, CAS #:237205)	5.0 grams
0.1mM EDTA (Invitrogen, Cat #: AM9912)	186 mg
Deionized water	500 mL

* dilute the buffer to 1x before use.

4. Cell counting

- Trypan Blue Solution, (0.4% Sigma, Lot #:RNBJ6324)
- Hemocytometer (BRAND™, 719520; Bürker Türk Counting Chamber)

Before you start

1 All steps should be performed in a sterile environment.
Prepare all the buffers and reagents at room temperature as described in the "Materials" section.

2 Freshly prepare the tissue dissociation solution.

Dilute the 10x RBCs lysis buffer to 1x.

3 Collect the tissue in a coulter counter cup filled with 15ml cold PBS (1x). Keep the tissues on ice before starting.

Preparation of single-cell suspension

4 Directly proceed by transferring the biopsies to a petri dish using sterile tweezers.

5 Cut the biopsies using a scalpel into smaller pieces at room temperature.

6 Transfer the pieces to the FACS tube containing 1ml of tissue dissociation solution (HBSS +1%P/S + Collagenase D +

DNaseI). Rinse the petri dish with tissue dissociation solution to collect all the materials.

7 Incubate for 1 hour @37°C, shake regularly. After this incubation keep the samples on ice.

8 Strain the mixture through a 70µm filter and collect the filtrate in a 50ml tube while keeping the samples on ice.
Push the sample through the filter using the back of a plunger.
Rinse the FACS tube with 1 ml cold HBSS+1%P/S and add it to the filter.
Wash the filter with 1 ml HBSS+1%P/S. Collect any remaining cell suspension materials from the back of the filter using a pipette.

9 Centrifuge @590xg, 5 min at 4°C .

10 Resuspend the pellet in 500 µL resuspension buffer (PBS+0,04% BSA) and transfer the sample to a 1.5ml Low-Bind tube. Add 1 ml RBCs lysis buffer (1x) and incubate for ~2-5 minutes on ice.

11 Centrifuge @590xg, 5 min at 4°C.

12 Resuspend the pellet in 50µL resuspension buffer (PBS+0,04% BSA).

13 Count the cells using Trypan blue staining (1:1 ratio of Trypan blue to sample) and a manual hemocytometer.
While keeping the rest of the cells on ice.

13.1 Proceed with 10x chromium protocol if 80-90% of the cells are alive.

10x single-cell RNA-seq kit instructions

14 Continue with 10x Chromium (Next GEM Single Cell 3′ reagent kit) and load 10,000 cells on a single lane.