Dissociation of Single Cell Suspensions from Human Breast Tissues

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dx.doi.org/10.17504/protocols.io.t3geqjw

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Oct 19, 2018

Working

ABSTRACT

This protocol was developed for the human breast cell atlas (HBCA) project to obtain high-viability cell suspensions from freshly dissociated breast tissues from human patients. There are two options for performing this protocol: *rapid-dissociation* (15-30 min) or *exhaustive dissociation* (overnight). In our experience the rapid dissociation protocol will provide higher cell viability and better representation of breast cell types, however the total number of cells dissociated may be lower after enumeration. In contrast the exhaustive protocol provides higher cell numbers and maintains good cell viability but may result in skewing of cell type representations over the long time period. The rapid-dissociation protocol was used to generate most of the cell suspensions for the MD Anderson human breast cell atlas project and has been optimized and tested more extensively.

TAGS

Single-cell

breast

NL_Breasttissue dissociation_protocol_v2.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Flowmi™ Cell Strainer 40 µm	H13680-0040	Bel-Art
Ultrapure BSA	AM2616	Ambion
10x MACS RBC lysis buffer	130-094-183	Miltenyi Biotec
Collagenase A	11088793001	Sigma
DMEM F12/HEPES	113300	Gibco - Thermo Fischer
DMEM medium	D5796	Sigma
BSA fraction V	15260037	Gibco - Thermo Fischer
Cell strainer 70um filter	352350	Falcon

MATERIALS TEXT

Consumables:

- Scalpels, 1.5ml tubes, 50ml conical, syringe, PBS.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

Making 10xcollagenase solution

1 Dissolve **0.04 g collagenase A** in **4 ml DMEM F12/HEPES media** .

Dissociation solution (DS)

2 **4 ml 10xcollagenase DMEMF12/HEPES**

26 ml DMEMF12/HEPES

10 ml BSA fraction V

Mincing

3 In a 10 cm dish, place a 1-3cm^3 tissue section and add ~ **5 ml DS** . Mince the tissue with scalpels into 1 mm^3 pieces.

4 Transfer minced tissue to a 50 ml conical tube. Q.S. to 35 ml with DS, enough to cover all the tissue.

Dissociation

5 Two options: ***Rapid-dissociation*** or ***Exhaustive Dissociation***

step case

Rapid-dissociation

no description provided

6 Gently dissociate the suspension at **37 °C** in a hybridization oven for **00:15:00** - **00:30:00** .

NOTE

With rotator set to medium (optional: pipet up and down with a 5 ml pipette to aid digestion during the incubation period).

7 Place the 70 µm filter on top of a 50 ml conical tube and filter the tissue suspension. Use a syringe plunger flange to grind the leftover unfiltered tissue. Rinse the filter with DMEM to ensure any remaining single cells are filtered. If necessary, re-filter the solution one more time.

Spin

8 Spin at 430 xg for **00:05:00** .

Remove the top adipocyte layer and the rest of the supernatant.

9 NOTE
The adipocyte layer can be saved for future analysis by storage in 90% DMSO and 10% FBS. However, the adipocyte cells are usually larger than 50 microns and can therefore not be loaded into the 10X genomics microfluidic chips.

Resuspending

10 Resuspend the cell pellet in 1 ml DMEM .

11 Pass cell suspension through a 40 µm flowmi filter to a 1.5 ml tube.

Centrifugation

12 Centrifuge cells at 4 °C 300 xg for 00:05:00 . If no RBCs are visible, skip directly onto step 19.

RBC lysis

13 Remove the supernatant and resuspend the cell pellet in: 1x MACS RBC lysis buffer.

NOTE
RBC lysis can be done up to 2 times. You can check the cells under a light microscope to see if RBCs are still present after each lysis step.

14 Incubate nutating at RT for 00:10:00 in 10 ml - 20 ml 1X buffer .

Centrifugation

15 Add 2X the volume of DMEM into the tube and centrifuge cells at 4 °C 300 xg for 00:05:00 .

16 Remove the supernatant and resuspend the cell pellet in 1 ml cold PBS .

17 Filter with a 40 µl flowmi into a 1.5 ml tube.

18 Spin at 300 xg for 00:05:00 .

Wash

19 After centrifugation, remove the supernatant and wash cells with **1 ml cold PBS** .

20 Centrifuge cells at **4 °C** 300 xg for **00:05:00** .

21 Remove the supernatant.

For 10X genomics RNA experiments

22 Resuspend cell pellet in PBS+ 0.04% BSA. Adjust cell concentration to 700-1200 cells/µl.

23 To check for **nucleated cells**: Make a 1:1 solution of Hoechst and PI, pipette **2 µl** of this mix to **10 µl filtered cells** and count in the Countess II FL (ThermoFisher) or a Hemocytometer.

24 To check for **viability**: Mix **10 µl Trypan blue** + **10 µl cells** . Load **10 µl of the cell suspension** for counting using a Hemocytomer or the Countess II (ThermoFisher)

Dissociation

step case

Exhaustive Dissociation

no description provided

6 Gently dissociate the suspension at **37 °C** in a hybridization oven for **12:00:00** .

NOTE
With rotator set to medium (optional: pipet up and down with a 5 ml pipette to aid digestion during the incubation period).

7 Place the 70 µm filter on top of a 50 ml conical tube and filter the tissue suspension. Use a syringe plunger flange to grind the leftover unfiltered tissue. Rinse the filter with DMEM to ensure any remaining single cells are filtered. If necessary, re-filter the solution one more time.