VERSION 1

OCT 17, 2019

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.7m9hk96

Protocol Citation: Samah El Ghamrasni 2019. Breast tumours dissociation .

protocols.io

<https://dx.doi.org/10.17504/protocols.io.7m9hk96>

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

Created: Sep 24, 2019

Last Modified: Oct 17, 2019

PROTOCOL integer ID:
28065

Keywords: Breast, single-cell RNAseq, tissue dissociation

Breast tumours dissociation V.1

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ABSTRACT

A protocol designed to dissociate fresh breast tissues (surgical specimens and biopsies) for single-cell RNAseq.

The protocol has been demonstrated to work successfully with fresh and cryopreserved tissues.

GUIDELINES

Requires access to a flow sorter

MATERIALS

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BSA Contributed by users

TrypLE Contributed by users

Gibco Penicillin-Streptomycin (10,000 U/mL) (Pen/Strep) Fisher Scientific Catalog # 15-140-122

FBS Invitrogen - Thermo Fisher

Liberase TL Roche Catalog #05 401 020 001

DMEM Gibco - Thermo Fischer Catalog #11885

PBS Contributed by users

SYTOX™ Blue Dead Cell Stain, for flow cytometry Thermo Fisher Catalog #S34857

Base Media: DMEM + Penstrep + 10%FBS

Resuspension buffer: PBS+0.01%BSA

Tissue Dissociation

- 1 Transfer the tissue onto a 10 cm petri dish
- 2 Rinse 1x briefly with ice cold PBS and aspirate PBS off.
- 3 Use a blade to carefully cut the sample into small pieces, approximately 3-4 mm in diameter.
- 4 Transfer pieces into 50ml tube and Resuspend in 5ml of Base media + Liberase (200ug/ml)
- 5 Incubate 2 hours at 37C
- 6 Mix with a 5 ml serological pipet 5 times to break up the pieces.
- 7 Let the pellet settle at the bottom of the tube, and transfer supernatant to a new falcon tube (Filter supernatant using 40um mesh)
- 8 Resuspend remaining tissue in 2mL of TrypLE, incubate for 10 min at 37C

- 9 Repeat Step 6 and 7
- 10 Spin cells down (300g for 10min at 4C) and Resuspend cells in resuspension media
- 11 Count cells

Flow Sorting

- 12 Stain cells with SytoxBlue (1ul/ml)
- 13 Incubate at room temperature for 15 min
- 14 Transfer to ice and proceed to flow sorting