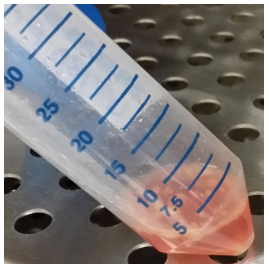
Oct 17, 2019 Version 1

Breast tumours dissociation V.1



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Samah El Ghamrasni[1]

[1]Princess Margaret Cancer Research Centre

Human Cell Atlas Metho... CZI START Project

Samah El Ghamrasni
Princess Margaret Cancer Centre Research

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

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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

MATERIALS

- BSA
- TrypIE
- 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 Fisher Scientific Catalog #**11885
- PBS
- SYTOX™ Blue Dead Cell Stain, for flow cytometry **Thermo Fisher Catalog #**S34857

Base Media: DMEM + Penstrep + 10%FBS
Resuspension buffer: PBS+0.01%BSA

Troubleshooting

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 TrypIE, 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