Human Breast Tissues Dissociation

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Human Cell Atlas Method Development Community

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Working
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PROTOCOL STATUS

Working
We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Collagenase Type I powder	17100017	Thermo Fisher Scientific
DMEM and Hams F-12 50/50	MT10090CV	Fisher Scientific
Deoxyribonuclease I	D4263-5VL	Sigma Aldrich
Dulbeccos Phosphate-Buffered Salt Solution 1X	MT21031CV	Fisher Scientific
0.05% Trypsin	MT25051CI	Fisher Scientific
Antibiotic-Antimycotic	15240062	Thermo Fisher Scientific
Fetal Bovine Serum	FB-12	Omega Scientific. Inc.

BEFORE STARTING
Prepare 4mg/mL collagenase solution before start and place on 37C shaker to dissolve.

Prepare materials

1

Collagenase Solution
100mg/mL Collagenase Type I in DMEM/Ham's F12

Media
DMEM/Ham's F12
10% FBS
1% Antibiotic

DMEM/Ham's F12
5% FBS
1% Antibiotic

DNAse
1mg/mL DNase in PBS

Initial Tissue Preperation

2

Transfer tissues to 150cm plate.
Wash with large quantities of PBS to remove blood and old storage media.
-Ammount of PBS varies depending on vloume of tissues washed.
Aspirate off PBS.

Repeat PBS wash twice, for a total of three washes.

Collect small pieces of tissues for FFPE or OCT Blocks.

Mechanical Dissociation of Tissue

3

Use two number 10 scaple to chop tissue.
Chop tissue until epithelial tissues are about 2-3mm in size.
-Depending on stating tisues sample, removal of excessive adipose tissues will help in the chopping and digesting process.

Enzymatic Dissociation of Tissue

4

Transfer anout 20mL in volume of chopped tissue to each 50mL concial tube.
Add 20mL of DMEM with 5% FBS with 4mg/mL Collagenase Type I.
Place on 37C shaker at about 180-200rpm.
Leave on heated shake for 12-16 hours.

Organoid Collection

5

Centrifuge tubes at 400gx5min.
Aspirate to remove supernatant.
Wash with 50mL of PBS.
Centrifuge at 400gx5min to remove PBS.

Single Cell Preperation

6

Add 2mL of 0.05% Trypsin to pellet of organoids.
Place at 37C for 6 minutes.
Take tube out every 2 minutes to pipette up and down with P1000, for a total of three times.

Add 10mL of DMEM with 10% FBS to tube.
Centrifuge at 400gx5min.
Aspirate off supernatant.
Resuspend pellet in 1mL of DMEM with 10% FBS
Add 100uL of DNase, incubate for 5min.
Add 10mL of DMEM with 10% FBS.
Centrifuge at 400gx5min
Remove supernatant
Resuspend in 10mL of DMEM with 10% FBS
Count cells.
Centrifuge at 400gx5min.
Bring back up to appropirate concentration for FACS staining.

Single Cell FACS Preperation

7

Prepare appropirate tubes for FACS staining.
To all tube add the following antibidies.
-PE-CD49f
-APC-EpCAM
-DAPI-CD45/CD31
--Use antibody concentraion suggested by manfacture.

Stain for 20min at 4C
Take out and wash cells with DMEM.
Pass through FACS tube filter.

Before sort begins add SytoxGreen to tube.

Sort for all live cells, anything that is SytoxGreen negative.