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Dissociation of fresh colorectal biopsies

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

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33084

ABSTRACT

Current procedures for the dissociation of intestinal biopsies (<50mg)

GUIDELINES

This approach is routinely applied to both pre-adenoma and normal intestinal tissues smaller than 50mg. Tissue should be reduced to <50mg or the volumes should be scaled up when accomodating larger tissues.

Tissue should be kept at 4°c and processed immediately on receipt.

We start by chelating the tissue at 4°c for 1hr 15min with rotation to loosen the epithelium from the mucosa.

Then the tissue is moved to a cold protease solution for dissociation, also at 4°c with rotation, usually for 25 minutes. During dissociation, we pipette the tissue gently at 10 and 20 minutes with a pipette tip large enough to allow the tissue to pass easily. At 25 minutes (or when the tissue begins to soften and yield significant material) we then pipette it more aggressively with a normal p-1000 tip for 5-10 passes to break it apart.

Finally, given a sufficient cell yield, we pass the solution through a 70um filter to remove remaining tissue and begin washing the resulting cells. Washing ends with a second filtration just before final dilution and encapsulation.

MATERIALS

MATERIALS

1M DTT Teknova Catalog #D9750

Protease from Bacillus Licheniformis Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5380

DNase Merck MilliporeSigma (Sigma-Aldrich) Catalog #DN25-100MG

DPBS without calcium & magnesium Corning Catalog #21-031-CV

EDTA 0.5M pH 8 Corning Catalog #46-034-CI

mini-strainer 70µm pluriSelect Catalog #43-10070-50

SAFETY WARNINGS

- ⚠ Pipetting and transfer of human derived cell-solutions carries risk of exposure. Use PPE and appropriate precautions. If cutting tissue, use an approved safety blade/scalpel and properly dispose of it (biohazardous sharps).

BEFORE START INSTRUCTIONS

Prepare and freeze aliquots of 2x DNase and 20x protease.

Prepare chelation buffer day-of procedure/collection.

Ensure that cold PBS, ice buckets, waste collection, etc. are all available.

Cool centrifuge (before reaching cell washes)

Prep work

1 Hydrate, aliquot, and freeze 2x DNase

DNase Merck MilliporeSigma (Sigma-Aldrich) Catalog #DN25-100MG

DPBS without calcium & magnesium Corning Catalog #21-031-CV

Hydrate to 5mg/ml in DPBS; keep cold; aliquot 1ml each into 2ml tubes and freeze immediately. Store at -20°C and use within 6 months.

2 Hydrate, aliquot, and freeze 20x Cold protease

Protease from *Bacillus Licheniformis* Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5380

Hydrate to 100mg/ml in molecular grade H₂O; keep cold; aliquot 100ul each into 2ml tubes and freeze immediately.

Store at -20°C and use within 6 months.

3 Dilute to 0.1M, aliquot, and freeze stock DTT

1M DTT Teknova Catalog #D9750

Dilute the 1M stock to 0.1M by adding 1 part 1M DTT to 9 parts Molecular bio grade H₂O. Mix well and aliquot at 1-2ml each. Freeze and store at -20°C for up to 6months. Use 0.1M aliquots within 1 week of thawing.

4 On the day of tissue receipt, prepare chelation buffer:

DPBS	49.35 ml
0.5M EDTA	400µl
0.1M DTT (diluted from 1M stock)	250µl

DPBS without calcium & magnesium Corning Catalog #21-031-CV

EDTA 0.5M pH 8 Corning Catalog #46-034-CI

Recipe / Grossing

5m

- 5 Upon receipt, inspect tissue and record information such as:
 - Accession # and other relevant collection metadata
 - Size - this can be as simple as drawing a spot the size of the tissue with description (flat, round, etc.)
 - Appearance
 - Presence of blood
 - Weight - in the interest of moving forward and not drying out a sample, we do not weigh tissue. Our samples are typically 20 - 40mg or less

5.1 If tissue is solid/pedunculated, it may need to be bisected or trisected to increase surface area, do not cut smaller than 2mm².

6 Wash tissue gently in DPBS to remove any debris or excess transport media.

Chelation

1h 15m

7 Move tissue to a 2ml tube containing ~1.8ml of chelation buffer and place on a tube rotator with continuous rotation. 1h 15m

Equipment

Labquake Rotisserie

NAME

Tube Rotator

TYPE

Thermo Scientific

BRAND

TS-4002110Q

SKU

7.1 Gently replace chelation buffer on sample at 30 minutes and 1 hour into chelation, ensuring that enough air remains in tube to displace (agitate) tissue during rotation

7.2 Prepare DNase for use before chelation is over by adding 1ml of DPBS to a frozen 1ml aliquot of 2x DNase and leaving on bench to thaw. Move DNase to Ice after it has thawed.

Dissociation

25m

- 8 Gently remove chelation buffer and re-suspend tissue in DPBS.
- 9 Quickly prepare Dissociation solution by adding 1.9ml of 1x DNase to a frozen 100µl aliquot of 20x cold Protease. Mix by pipetting.
- 10 Gently remove DPBS from tissue and resuspend in 1.8ml of Dissociation solution. Make sure there is enough air in the tube to displace the tissue on inversion.
- 11 Place on a tube rotator with continuous rotation.

25m

Equipment	
Labquake Rotisserie	NAME
Tube Rotator	TYPE
Thermo Scientific	BRAND
TS-4002110Q	SKU

- 11.1 At 25 minutes (end of dissociation) triturate using a normal p-1000 tip 5-10 times, such that tissue is deformed as it enters the tip to yield material into the supernatant, and clusters are broken apart. The supernatant should become somewhat cloudy with cells. Inspect supernatant on a hemocytometer. Cells should be mostly single or in clusters of 10 or less. There should be at least 100K cells total. If tissue does not adequately yield cells, continue incubation for another 5 minutes before triturating again.

Final washes

15m

- 12 Pipette cells to mix and re-suspend before transferring onto a 70µm filter on a clean tube in a cold rack. Allow cells to filter through by gravity and check filtrate for cells before proceeding.

mini-strainer 70µm pluriSelect Catalog #43-10070-50

- 13** Spin cells at 700xg for 5 minutes to pellet.

700 rcf, 4-6°C,
00:05:00

5m

- 14** Ensure that cells have visibly pelleted before removing supernatant. Resuspend in DPBS using a wide-bore p-1000 tip. If cells do not pellet, you may need to increase speed by 100xg. As cells continue to die and secrete material, you may observe stringy aggregates in the supernatant or pellet. Ideally this material remains in the supernatant and is removed during washes, however if persists and becomes too large it may prevent cells from pelleting.

- 15** Repeat the previous 2 steps (wash) twice, checking cell number and quality upon resuspension.

- 16** On the 3rd resuspension into DPBS (or single cell running buffer), gently resuspend cells at roughly 2x the desired final concentration or greater and pass through a 70µm flowmi filter into a clean tube.

Flowmi Cell Strainer 70µm Bel-Art

- 17** Check cells, dilute as needed and proceed to encapsulation.