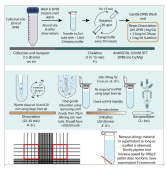

Aug 03, 2021

Dissociation of fresh colorectal biopsies

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dx.doi.org/10.17504/protocols.io.bci4iugw

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ABSTRACT

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

DOI

dx.doi.org/10.17504/protocols.io.bci4iugw

PROTOCOL CITATION

Alan J Simmons, Ken Lau 2021. Dissociation of fresh colorectal biopsies. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bci4iugw

CREATED

Feb 13, 2020

LAST MODIFIED

Aug 03, 2021

PROTOCOL INTEGER ID

33084

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 reciept.

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 sufficuent 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 TEXT

MATERIALS

1M DTT

Teknova Catalog #D9750 Step 3

Protease from Bacillus Licheniformis

Sigma Catalog #P5380 Step 2

DNAse **Sigma**

Aldrich Catalog #DN25-100MG Step 1

DPBS without calcium &

magnesium **Corning Catalog #21-031-CV** In 2 steps

EDTA 0.5M pH

8 **Corning Catalog #46-034-CI** Step 4

mini-strainer

70µm **pluriSelect Catalog #43-10070-50** Step 12

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 STARTING

Prepare and freeze aliqots 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 **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

Sigma 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.

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

DPBS	49.35ml
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**

Reciept / Grossing 5m

5 Upon reciept, 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 $2mm^2$.

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

Labquake Rotisserie
Tube Rotator
Thermo Scientific TS-4002110Q

7.1 Gently replace chelation buffer on sample at 30 minutes and 1 hour into chelation, ensuring that

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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 aliqout of 2x DNAse and leaving on bench to thaw. Move DNAse to Ice after it has thawed.

Dissociation 25m

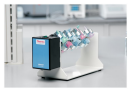
8 Gently remove chelation buffer and re-suspend tissue in DPBS.

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

Labquake Rotisserie
Tube Rotator
Thermo Scientific TS-4002110Q



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 shoud 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 transfering 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. 5m

700 rcf, 4-6°C, 00:05:00

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

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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.

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