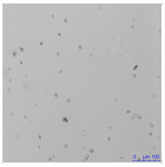

Version 2

Feb 26, 2021

Isolation of live single cells from intestinal biopsy V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bst7nern

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol describes dissociation of a human intestinal biopsy tissue into single cells, followed by depletion of dead cells via annexin V MACS beads. The outcome is a single-cell suspension with viability ≥ 90% that is used for single-cell sequencing or establishemt of enteroid/colonoid culture.

DOI

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PROTOCOL CITATION

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WHAT'S NEW

Longer enzyme incubation time to reduce cell clusters

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47711

MATERIALS TEXT

Dulbeccos Phosphate Buffered Saline **Thermo Fisher Scientific Catalog #14040133**

Fetal Bovine Serum, certified, United States **Thermo Fisher Catalog #16000036**

Bovine Serum Albumin **Sigma Aldrich Catalog #A3059-500G**

HBSS, no calcium, no magnesium, no phenol red **Thermo Fisher Catalog #14175079**

Liberase TH **Millipore Sigma Catalog #5401135001** : for a stock solution of 13 Wunsch units (WU)/mL (2.5 mg collagenase/mL), reconstitute 1 vial in 2 mL sterile HBSS, store 100uL aliquots at -20oC. After thawing one 100uL tube, make 15uL aliquots and freeze. After thawing a 15uL aliquot, discard the leftovers and don't refreeze.

DNAse I **Roche Catalog #10104159001** : reconstitute 100mg in 4mL sterile PBS, yielding a 50U/mL solution (100x). Aliquot 100uL/tube and store at -20oC

Scissors Iris **Fine Science Tools Catalog #14058-09**

Dead Cell Removal Kit **Miltenyi Biotec Catalog #130-090-101**

LS Columns **Miltenyi Biotec Catalog #130-042-401**

MidiMACS Separator **Miltenyi Biotec Catalog #130-042-302**

MACS MultiStand **Miltenyi Biotec Catalog #130-042-303**

ThermoMixer
Eppendorf 05-412-501

Cell culture plasticware:
- 1.5mL eppendorf tubes
- 50mL conical tubes
- 15mL conical tubes
- 100µL cell strainers
- 30mm round culture dish
- Barrier pipette tips
- Serological pipetes

Before you begin 10m

1 Prepare reagents:
- **5 mL** Cryo-wash (4.5mL PBS + 500µL FBS)

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- **1.5 mL** Enzyme Mix (30µL Liberase TH stock + 30µL DNAse I stock + 3mL HBSS)
- **10 mL** Blocker Buffer (4% BSA in PBS: reconstitute 400mg BSA in 10mL PBS, filter-sterilize)
- **2 mL** Cell Reconstitution Buffer (2mL Advanced DMEM:F12 + 50µL Blocker Buffer + 20µL DNAse I)
- **20 mL** 1x MACS Binding Buffer (1mL 20x concentrate + 19mL sterile ddH2O*)

**Do not substitute with deionized water (this will impair annexin V binding)*

Recover the cryopreserved tissue 7m

2 ⚠

Thaw the tissue quickly in a **37 °C** water bath

If using a bead bath, put a beaker with pre-heated water into the beads and place the cryovial in the water

3 As soon as the vial contents are liquified, transfer the tissue suspension to an eppendorf tube

We finely mince the biopsy tissue before cryopreservation. If you are working with a biopsy that was cryopreserved whole, ***do not skip step 6***

4 Spin the tube 30s - 700g , discard the supernatant
700 x g, 00:00:30 **Room temperature**

5 Wash the tissue (repeat 3 times)

5.1 Resuspend the pellet in **1 mL** Cryo-wash

5.2 Spin the tube 30s - 700g, discard the supernatant
700 x g, 00:00:30 **Room temperature**

6 ⊛

Skip this step if biopsy was minced before cryopreservation

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6.1 Reconstitute the washed tissue in **500 µl** Cryo-wash supplemented with **5 µl** DNAse I

6.2 Mince the tissue inside the eppendorf tube using sterile surgical scissors

6.3 Spin the tube 30s - 700g, discard the supernatant
700 x g, 00:00:30 **Room temperature**

Single cell dissociation 1h

7 Resuspend the pellet in **1 mL** Enzyme Mix

8 Incubate in a thermomixer 37°C - 15m, 800RPM
800 rpm, 37°C

8.1 During digestion, place a 100µm strainer in a 50mL conical and pre-wet the membrane with with **5 mL** Blocker Buffer

9 After digestion, let the tissue fragments settle by gravity to the bottom, pass the supernatant through prepared strainer from step 8.1

10 Repeat the digestion (steps 7-9) with fresh Enzyme Mix 2 more times (3 cycles of 15m total)

11 After the final digest, pass the tissue fragments 10 times through a P1000 tip

12 Pass the digested suspension through the strainer:

12.1 Apply the suspension from step 11 directly to the strainer membrane

12.2 Take the strainer out of the conical tube and place it in a round 30mm TC dish

A larger size dish can be used in lieu of a 30mm dish

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12.3 Use the flat surface of an insulin syringe plunger to force the remaining undigested fragments through the strainer by rubbing the plunger in a circular motion, discard the plunger

12.4 Rinse the strainer by lifting it slightly from the round dish and applying **2 mL** Blocker Buffer, capturing the flow-through in the 30mm dish; keep the strainer suspended

12.5 Rinse the strainer bottom with **2 mL** Blocker Buffer, capturing it in the 30mm dish

12.6 Transfer the suspension from the 30mm dish to the 50mL conical from step 8.1

12.7 Rinse the 30mm dish with **1 mL** Blocker Buffer and transfer to the same conical

13

Transfer the suspension from 50mL conical to a new 15mL conical.

This step is optional, but recommended, because it results in a more visible pellet than when spinning in a 50mL conical

14 Spin down the cells 5m - 500g, resuspend in **1 mL** Cell Reconstitution Buffer

500 x g, 00:05:00 **Room temperature**

15 Strain the suspension into a FACS tube through the 35µm strainer cap

It is essential to strain the cells before proceeding to column-based dead cell removal

16 Count the cells, noting the viability

Dead cell removal 30m

17 Pellet the cells: 5m - 500g, discard the supernatant

500 x g, 00:05:00 **Room temperature**

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18 Resuspend the pellet in **200 µl** of MACS Dead Cell Removal MicroBeads

19 Incubate 15m - room temperature
00:15:00 **Room temperature**

19.1 During the incubation, prepare the MACS LS column by placing it into the magnet and rinsing with **3 mL** MACS Binding Buffer (discard the flow-through)

20 Add **300 µl** MACS Binding Buffer to the suspension from step 19 to total volume of **500 µl**

21 Apply the suspension to the prepared MACS LS column (in the magnet) and collect the flow-through into a 15mL conical tube

The flow-through contains live cells, while dead cells are captured in the column

22 Wash the column 4 times with **3 mL** MACS Binding Buffer, keep collecting the flow-through (live cells) into the same tube.

At this point the column can be discarded, unless you are troubleshooting and intend to elute the captured dead cells.

23 Spin the live cell suspension 5m - 500g, discard the supernatant
500 x g, 00:05:00 **Room temperature**

24 Resuspend the pellet in **500 µl** Cell Reconstitution Buffer

25 Count the cells, noting the viability

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