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Isolation of live single cells from intestinal biopsy

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Works for me

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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 $\geq 90\%$ that is used for single-cell sequencing or establishment of enteroid/colonoid culture.

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MATERIALS TEXT

 [Dulbecco's 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](#)

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

Cell culture plasticware:

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



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 $\geq 90\%$ that is used for single-cell sequencing or establishment of enteroid/colonoid culture.

Before you begin

10m

1 Prepare reagents:


-  **5 mL** Cryo-wash (4.5mL PBS + 500μL FBS)
-  **1.5 mL** Enzyme Mix (15μL Liberase TH stock + 15μL DNase I stock + 1.5mL 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 (1 mL 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

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

45m

7 Resuspend the pellet in  **500 µl** Enzyme Mix

8 Incubate in a thermomixer 37°C - 10m, 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 10m 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

- 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

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