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Human intestinal cell dissociation suitable for multi-omics single-cell assays

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1 Works for me Share dx.doi.org/10.17504/protocols.io.bubrnsm6

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ABSTRACT

We developed a protocol for the preparation of a single-cell suspension of human intestinal (ileal) biopsies, optimized for contemporary multi-omics single-cell assays including high-dimensional flow cytometry and single-cell RNA sequencing (scRNA-seq) assays.

The protocol provides a rapid and efficient method of digesting human intestinal biopsies into a single-cell suspension resulting in a high viable cell yield and preservation of cell-surface markers. Notably, the protocol utilizes a Collagenase-I/Benzonase-based 37°C dissociation method, which collects already digested cells at several steps during the process to prevent over-digestion (fractioning technique).

We obtained a total cell yield of ~5.8E6 +/- 1.9E6 cells, with a viability of 71% +/- 6% (n=9) (Figures 1A and 1B) from biopsy weights ranging 10-14 mg. By flow cytometry, all major immune cell lineages were detected, as well as a CD45-/EpCAM+ epithelial cell population (20%-60% of total viable singlets; Figure 1C). scRNA-seq analysis (10X Genomics; 5'/VDJ with feature barcoding) showed high-quality viable cells with low mitochondrial gene counts, indicating this protocol exerts minimal stress on the digested cells (Figure 1D).

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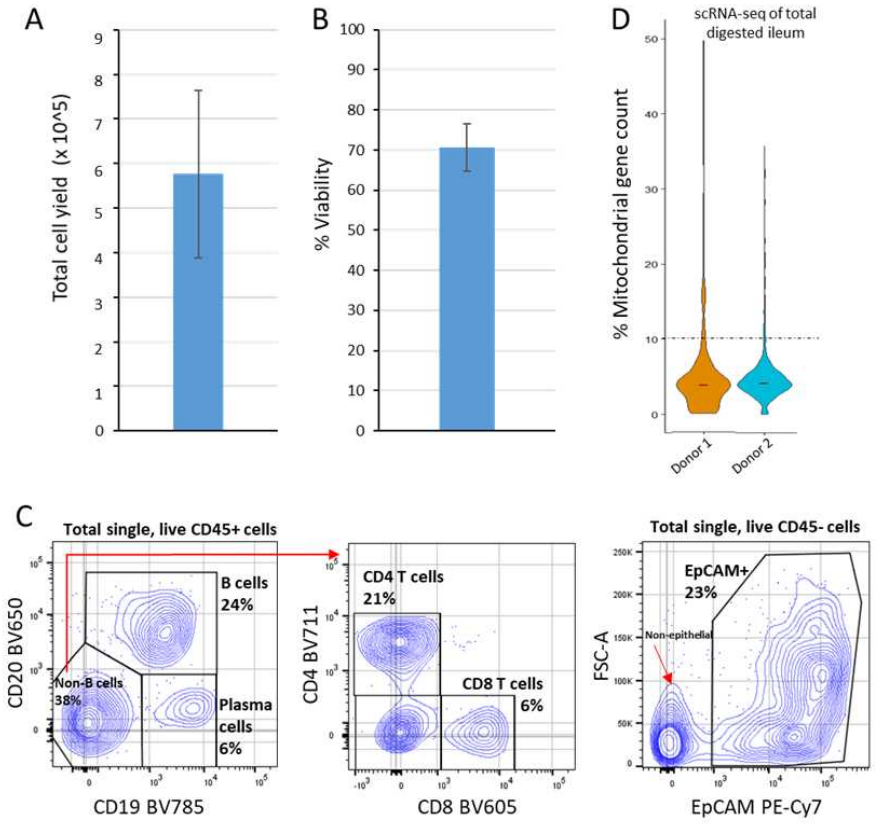

Figure 1. A) Total cell yield, and B) Percent viability measured using propidium iodide/acridine orange-based cell count (Nexcelom Cellometer K2). Bar graphs represent mean and standard deviation from 9 independent experiments. C) Flow cytometry plots showing cell-surface markers for B cell (CD20+, CD19+), T cells (CD4+, CD8+), and epithelial cells (CD45-, EpCAM/CD326+). To identify B and T cells, total single viable cells were gated to include total CD45+ cells, which were cleaned from myeloid cells followed by projection of CD19, CD20 (B cells), and CD4, CD8 (T cells). For epithelial cells, total single viable cells were first gated to exclude CD45+ immune cells, and then EpCAM was projected to reveal total epithelial cells within CD45- cells. The percentage shown on the flow plots represents the percentage of each cell subset within total CD45+ cells (B and T cells) or total CD45- cells (EpCAM+). D) Mitochondrial gene counts from 2 independent scRNA-seq experiments (10X Genomics platform).

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intestine, gut, ileum, tissue dissociation, flow cytometry, scRNA-seq, single-cell suspension, multi-omics, cell-surface protein, human, enzymatic digestion, collagenase-I

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GUIDELINES

- Keep all solutions on ice unless otherwise mentioned.
- Cells will be isolated into 3-4 different fractions based on amount of tissue being processed and pooled for downstream processing.

MATERIALS TEXT

- Cell counter (Nexcelom Cellometer K2)
- Cooled large tabletop centrifuge
- 37°C water bath
- gentleMACS™ (gMACS) Octo Dissociator (Myltenyi)

- Razor blade
- 6 cm Petri-dish
- 15 mL Falcon tubes
- 50 mL Falcon tubes
- 70 uM nylon filters (pluriStrainer for 15 mL #43-10070-40; for 50 mL #43-50070-51)
- Glass Pasteur pipettes & squeeze bulb
- C-tubes for gentleMACS™ (Mylteny #130-093-237)

- def-RPMI* (or RPMI-1640)
- Newborn Calf Serum (NBCS) or FBS
- Collagenase-I (0.25%) (Stem cell Technologies #07902)
- Benzonase (Sigma #E1014)
- 0.5 M EDTA pH 8.0
- DTT

Note: *def-RPMI is a custom media prepared in-house and composed of the same formulation of commercially available RPMI-1640, but deficient in the following compounds: biotin, L-glutamine, phenol red, riboflavin, and sodium bicarbonate. These compounds were removed to avoid interference with standard flow-cytometry assays.

Solution preparation: Keep all solutions on ice unless otherwise mentioned.

- **def-RPMI/3%NBCS:** def-RPMI + 3% NBCS (or RPMI-1640 + 3% FBS)
- **def-RPMI/3%NBCS/Benzonase**: Benzonase @ 1 uL per 10 mL def-RPMI/3%NBCS
- **def-RPMI/3%NBCS/EDTA**: EDTA @ 5 mM EDTA final concentration in def-RPMI/3%NBCS
- **def-RPMI/3%NBCS/EDTA/DTT**: DTT @ 15 mg per mL def-RPMI/3%NBCS/EDTA
- **Collagenase-I/Benzonase**: Benzonase @ 1 uL per 5 mL Collagenase-I (0.25%)

1 Weigh the biopsy tissue.

2 Chop tissue into small pieces with a razor blade in def-RPMI/3%NBCS/EDTA/DTT media in a 6 cm dish, on ice.

3 Transfer tissue solution to 15 mL conical (use 1 mL pipet).

4 Rinse the petri dish with additional media (same as in Step 2) and transfer to 15 mL tube from Step 3.

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5 Vortex the tissue solution, and incubate at RT for 5 min, vortexing intermittently.

6 Fill the 15 mL conical with def-RPMI/3%NBCS.

7 Spin for 5 min at 450 g at 4°C. **450 x g, 4°C, 00:05:00** 5m

8 Aspirate supernatant.

9 Resuspend pellet in 1 mL def-RPMI/3%NBCS.

10 Pass cell solution through 70 uM filter placed on 15 mL conical and continue at Step 11. ***SAVE 70 uM FILTER WITH REMAINING TISSUE CHUNKS–CONTINUE AT STEP 25 IN PARALLEL with STEP 11.**

11 Fill conical with flow through with def-RPMI/3%NBCS to 12 mL.

12 Layer with 100% NBCS. To layer, use a 9 inch Glass Pasteur pipette and squeeze bulb to fill up the pipette with as much 100% NBCS as possible. Place the pipette such that its tip is at the bottom of the sample tubes and carefully take off the bulb. Allow the 100% NBCS to slowly form a layer at the bottom of the tube. Carefully lift the pipette out of the tube and keep the tube upright to maintain the layers through centrifugation.

13 Spin for 5min at 450g at 4°C. **450 x g, 4°C, 00:05:00** 5m

14 Resuspend pellet in 250-300 uL Collagenase-I/Benzonase.

15 Incubate at RT for 3-5 min with intermittent vortexing.

16 Fill conical with def-RPMI/3%NBCS/EDTA.

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17 Spin tube for 5 min at 450 g and 4°C. **450 x g, 4°C, 00:05:00** 5m

18 Aspirate supernatant.

19 Resuspend pellet in 1 mL def-RPMI/3%NBCS.

20 Pass through 70 uM Filter placed on a 15 mL conical.

21 Fill flow through conical with def-RPMI/3%NBCS to 12 mL.

22 Layer flow-through solution with 100% NBCS at the bottom of the tube (See Step 12)

23 Spin for 5 min at 450 g at 4°C. **450 x g, 4°C, 00:05:00** 5m

24 Resuspend pellet in 250-300 uL def-RPMI/3%NBCS/Benzonase (Fraction 1).

25 * Remove remaining tissues from filter and place into gMACS C-tube with 1 mL Collagenase-I/Benzonase.

26 Incubate 5 min in a 37°C waterbath, with intermittent vortexing.

27 Fill gMACS C-tube with def-RPMI/3%NBCS/EDTA.

28 Spin for 5 min at 450 g at 4°C. **450 x g, 4°C, 00:05:00** 5m

29 Aspirate supernatant.

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30 Resuspend pellet in 1 mL def-RPMI/3%NBCS/Benzonase.

31 Pass through 70 uM filter placed on 15 mL conical and continue with Step 32. ****SAVE 70 uM FILTER WITH REMAINING TISSUE CHUNKS – CONTINUE AT STEP 36 IN PARALLEL with STEP 32.**

32 Fill conical with flow through with def-RPMI/3%NBCS to 12 mL.

33 Layer flow-through solution with 100% NBCS at the bottom of the tube (See Step 12).

34 Spin for 5 min at 450 g at 4°C. **450 x g, 4°C, 00:05:00** 5m

35 Resuspend pellet in 250-500 uL def-RPMI/3%NBCS/Benzonase (Fraction 2).

36 ****** Remove remaining tissues from the filter into gMACS C-tube with 1.5 mL Collagenase-I/Benzonase.

37 Incubate for 5 min at 37°C on gMACS ("37_LPDK program;" pre-programmed in gMACS).

38 Fill gMACS C-tube with def-RPMI/3%NBCS/EDTA.

39 Spin for 5 min at 450 g and 4°C. **450 x g, 4°C, 00:05:00** 5m

40 Aspirate supernatant.

41 Resuspend pellet in 1 mL def-RPMI/3%NBCS/Benzonase.

42 Pass through 70 uM filter placed on 15 mL conical.

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43 OPTIONAL: If there are larger tissue chunks remaining, repeat Steps 36-42, but instead run RT gMACS "intestine_program" at Step 37 until chunks are mostly dissociated (1-3x).

44 If any small chunks remain, use the back of a 1 mL syringe plunger to push any undigested chunks through the filter into the same conical. Rinse the back of the plunger over the filter with 2-5 mL def-RPMI/3%NBCS.

45 Fill conical with flow through with def-RPMI/3%NBCS.

46 Layer flow-through solution with 100% NBCS at the bottom of the tube (See Step 12).

47 Spin for 5 min at 450 g at 4°C. **450 x g, 4°C, 00:05:00** 5m

48 Aspirate supernatant.

49 Resuspend pellet in 250-500 uL def-RPMI/3%NBCS/Benzonase (Fraction 3; and Fraction 4 if Steps 36-42 are repeated).

50 Count the cells in the 3-4 fractions individually.

51 Pool the 3-4 fractions for final cell count/viability. The cells are ready for additional downstream experiments.

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