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 We use this protocol and it's working

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Human small intestine cell dissociation (on ice) V.4

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ABSTRACT

Protocol for human small intestine cell dissociation, performed on ice to reduce artifact gene expression.

ATTACHMENTS

[human Intestine cell dissociation protocol.pdf](#)

MATERIALS

MATERIALS

Please see Guidelines for required materials **Contributed by users**

BEFORE START INSTRUCTIONS

Checklist prior to beginning:

- Centrifuges, large and small, set to 4 C
- Make enzyme stock; place 2 tubes of enzyme on dry ice.
- Make 0.01% BSA/PBS (50 mL)
- Things you need: petri dishes, clean forceps, razor blade, pipets, 30 µM filters, timer.

Stock solution for enzyme

- 895 µL DPBS
- 5 µL 0.5 M EDTA (2.5 mM final)
- Add 100 µL enzyme (100 mg/mL) to 900 µL of enzyme stock to make 1X enzyme mix. Add 28 mg of tissue to each 900 µL of enzyme mix.

- 1 While excluding as much PBS as possible, **weigh out tissue** using Mettler.
- 2 After weighing out tissue, **transfer to petri dish** on ice and **mince tissue** using grinding motion with razorblade for 2-3 minutes.
00:02:00 Mince tissue
- 3 After tissue is minced finely, **add 1 mL enzyme mix per 28 mg of tissue** to the petri dish and pipet minced tissue + enzyme into eppendorf tube (on ice).
1 mL enzyme mix per 28 mg of tissue
- 4 **Start timer.** Leave tube on ice - **initially shake vigorously to break up the tissue**, 3-5x every 30-45 seconds for 5 minutes.
00:05:00
00:00:30
- 5 Now, when big chunks are broken up, shake every 1 minute while leaving on ice for **5 additional minutes (10 minutes total time)**.
00:05:00
00:01:00
- 6 **After 10 minutes total digest time**, triturate the digest mix 10X using p1000 set to 700 µL.
- 7 Continue shaking every minute for **5 additional minutes (15 minutes total time)**.
00:05:00
00:01:00
- 8 **After 15 minutes digest time, triturate digest mix again 10X** and spin digest mix at 90 G for 30 seconds at 4 °C.
00:00:30 Spinning
4 °C
- 9 Remove supernatant (80%) containing single cells and filter using 30 µM filter while leaving chunks on bottom; rinse filter with 10 mL PBS/BSA into 50 mL conical (on ice) to save single cells.

10 mL PBS/BSA

- 10 To residual chunks of tissue add additional 1 mL of enzyme (per 28 mg tissue).

1 mL enzyme (per 28 mg tissue)

- 11 Shake vigorously 3-4X every minute for **10 additional minutes (25 minutes total time)**.

00:10:00 Shaking.

00:01:00

- 12 Triturate again 10X using 1 mL pipet set to 700 μ L.

- 13 Continue to shake vigorously every minute for **5 minutes additional time (30 minutes total time)**.

00:05:00

00:01:00

- 14 Triturate again 10X and filter using the same 30 μ M filter and rinse with 10 mL PBS/BSA into the same 50 mL conical (on ice).

10 mL PBS/BSA

- 15 Divide flow-through into 2 15 mL tubes.

- 16 Spin 600 g for 5 minutes at 4 °C.

4 °C Spinning

00:05:00 Spinning

- 17 Carefully remove supernatant - re-suspend both pellets in 100 μ L total PBS/BSA in one of the 15 mL conicals.

100 μ L PBS/BSA

- 18 **Add** 700 μ L RBC lysis buffer to 100 μ L PBS/BSA (800 μ L total). Triturate 20X using 1 mL pipet.
- 700 μ L RBC lysis buffer
 - 100 μ L PBS/BSA
- 19 Incubate for 3 minutes on ice.
- 00:03:00 Incubation
- 20 Add 10 mL of PBS/BSA to 15 mL conical to dilute the RBC lysis buffer.
- 10 mL PBS/BSA
- 21 Spin 600 G for 5 minutes at 4 °C.
- 00:05:00 Spinning
 - 4 °C
- 22 Remove supernatant.
- 23 Briefly re-suspend cells in a small volume of PBS/BSA and check to ensure that there are no more RBCs present.
- 24 **Re-suspend** in 10 mL total PBS/BSA in the same 15 mL conical.
- 10 mL PBS/BSA
- 25 Spin 600 g for 5 minutes at 4 °C.
- 4 °C Spinning
 - 00:05:00 Spinning

Remove supernatant and re-suspend in a small volume of PBS/BSA to check cell concentration.

- 27 Analyze quantity and viability of cells using a hemocytometer with trypan blue: add 10 μ L of trypan blue to 10 μ L of cell suspension, mix by pipeting and pipet into hemocytometer; **for Chromium, make concentration to 1 million cells per mL. For DropSeq, make concentration to 100,000 cells/mL.**