Intestine cell dissociation V.3

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ABSTRACT

Protocol for human intestine cell dissociation.

MATERIALS

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 0.5M 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

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While excluding as much PBS as possible, weigh out tissue using Mettler.

After weighing out tissue, transfer to petri dish on ice and mince tissue using grinding motion with razorblade for 2-3 minutes.

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).

Start timer. Leave tube on ice - initially shake vigorously to break up the tissue, 3-5x every 30-45 seconds for 5 minutes.

Now, when big chunks are broken up, shake every 1 minute while leaving on ice for 5 additional minutes (10 minutes total time).

After 10 minutes total digest time, triturate the digest mix 10X using p1000 set to 700 µL.

Continue shaking every minute for 5 additional minutes (15 minutes total time).

After 15 minutes digest time, triturate digest mix again 10X and spin digest mix at 90 G for 30 seconds at 4°C.

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.

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

Shake vigorously 3-4X every minute for 10 additional minutes (25 minutes total time).
12 Triturate again 10X using 1 mL pipet set to 700 µL.

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

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

15 Divide flow-through into 2 15 mL tubes.

16 Spin 600 g for 5 minutes at 4 °C.

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

18 Add 700 µL RBC lysis buffer to 100 µL PBS/BSA (800 µL total). Triturate 20X using 1 mL pipet.

19 Incubate for 3 minutes on ice.

20 Add 10 mL of PBS/BSA to 15 mL conical to dilute the RBC lysis buffer.

21 Spin 600 G for 5 minutes at 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.

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24 **Re-suspend** in 10 mL total PBS/BSA in the same 15 mL conical.

25 Spin 600 g for 5 minutes at 4 °C.

26 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 μL of trypan blue to 10 μL 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**.