Intestine cell dissociation Version 2

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Abstract

Protocol for human intestine cell dissociation.

Citation: Andrew Potter Intestine cell dissociation. **protocols.io**
dx.doi.org/10.17504/protocols.io.nx8dfrw
Published: 21 Mar 2018

Before start

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.

Materials

✓ Please see Guidelines for required materials by Contributed by users

Protocol

Step 1.
While excluding as much PBS as possible, **weigh out tissue** using Mettler.

Step 2.

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

Step 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).

AMOUNT
1 ml Additional info: enzyme mix per 28 mg of tissue

Step 4.

Start timer. Leave tube on ice - **initially shake vigorously to break up the tissue**, 3-

5x every 30-45 seconds for 5 minutes.

Step 5.

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

Step 6.

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

Step 7.

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

Step 8.

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

TEMPERATURE
4 °C Additional info:

Step 9.

Remove supernatant (80%) containing single cells and filter using 30 µM filter while leaving chucks on bottom; rinse filter with 10 mL PBS/BSA into 50 mL conical (on ice) to save single cells.

AMOUNT
10 ml Additional info: PBS/BSA

Step 10.

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

AMOUNT
1 ml Additional info: enzyme (per 28 mg tissue)

Step 11.

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

Step 12.

Triturate again 10X using 1 mL pipet set to 700 µL.

Step 13.

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

Step 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).

AMOUNT
10 ml Additional info: PBS/BSA

Step 15.

Divide flow-through into 2 15 mL tubes.

Step 16.

Spin 600 g for 5 minutes at 4 °C.

TEMPERATURE
4 °C Additional info: Spinning

Step 17.

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

AMOUNT
100 µl Additional info: PBS/BSA

Step 18.

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

AMOUNT
700 µl Additional info: RBC lysis buffer
AMOUNT
100 µl Additional info: PBS/BSA

Step 19.

Incubate for 3 minutes on ice.

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

Step 20.

AMOUNT
10 ml Additional info: PBS/BSA

Step 21.

Spin 600 G for 5 minutes at 4 °C.

TEMPERATURE
4 °C Additional info:
Step 22.

Remove supernatant.

Step 23.

Briefly re-suspend cells in a small volume of PBS/BSA and check to ensure that there are no more RBCs present.

Step 24.

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

AMOUNT
10 ml Additional info: PBS/BSA
Step 25.

Spin 600 g for 5 minutes at 4 °C.

TEMPERATURE
4 °C Additional info: Spinning
Step 26.

Remove supernatant and re-suspend in a small volume to check cell concentration.

Step 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.**