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

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Human Cell Atlas Metho...

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Attachments

PDF

human Intestine cell...

49KB

Materials

MATERIALS

Please see Guidelines for required materials

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.

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 chucks 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 µL**.

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 µM 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 µL total PBS/BSA in one of the 15 mL conicals.
100 µL PBS/BSA

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

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.**