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Version 2

Mouse P1 Kidney Cold-Active Protease Single Cell Dissociation V.2

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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

Method used to derive single cell suspension from P1 mouse kidneys on ice, generating a cell suspension with greatly reduced artifact gene expresion changes and suitable for downstream analysis using 10x Chromium or DropSeq.



Guidelines

Storage Conditions of Reagents

Reagent	Storage Condition
DPBS (Thermofisher, 14190144)	4°C
0.5 M EDTA (Ambion, AM9260G)	room temp.
BSA (Sigma, A8806)	4°C
Protease from <i>Bacillus</i> <i>Licheniformis</i> (Sigma, P5380)	Store 100 µL aliquots (100 mg/mL) in DPBS at -80°C
DNAse 1 (Applichem, A3778)	Store 10 μL aliquots (250 U/10 μL) in DPBS at -80°C

Required Equipment

Equipment	Suppli er	Catalog no.
gentleMACS dissociator	Milteny i	130-093-235

The protocol workflow is as follows:

- A. Isolate Kidney
- B. Initial digestion: triturate on ice
- C. Perform gentleMACS
- D. Continue triturating on ice
- F. Preparing cells for Chromium/DropSeq



BEFORE STARTING

Prepare Bacillus Licheniformis enzyme mix just prior to starting dissociation:

Volume (μl)	Reagent	Final concentration
894	DPBS	1X
1	0.5 M EDTA	0.5 mM
5	DNAse 1 (250 U/10 μL)	125 U / mL
100	<i>B. Lich</i> (100 mg/mL)	10 mg/mL

⁺²⁵ mg tissue / 1 mL enzyme mix

To prepare 0.01% BSA/PBS:

Make stock of 10% BSA in DPBS and store at -20 °C. To make PBS/BSA 0.01% aliquot 50 mL of DPBS in 50 mL conical and pipet in 50 μ L of 10% BSA stock.

Prepare 10% FBS/PBS with heat-inactivated FBS.

Troubleshooting

- 1 Extract & isolate P1 kidneys in ice-cold PBS.
- 2 Mince kidneys on top of petri dish, on ice, using razor blade.
- Weigh out 25 mg of tissue for each tube of B. Lich. enzyme mix (2 tubes total).

- 4 Incubate tissue + enzyme on ice for 7 minutes while triturating 15 strokes using 1 mL pipet every 2 minutes set to 700 μL first with tip cut off.
 - **(:)** 00:07:00
 - **(5)** 00:02:00
- 5 Monitor digestion by taking small aliquot and visualizing under scope (every 5 minutes).
 - **(5)** 00:05:00
- After 7 minutes, take the digest mix (combine the two tubes) and pipet into Miltenyi C-tube (placed on ice); take C-tube to gentleMACS placed in 4° cold room. Run program brain_03 two times.
 - **4** °C
- After MACS, briefly quick spin the MACS tube (to 500 G) at 4 °C to ensure contents are in the bottom of the tube.
 - **₿** 4 °C
- Re-suspend and visualize cells using scope by taking small aliquot and using a slide; continue digesting cells in C-tube on ice for 8 additional minutes while triturating every 2 min 15 strokes using a 1 mL pipet.
 - **©** 00:08:00
 - **(5)** 00:02:00
- 9 Add 3 mL ice-cold 10% FBS/PBS to digest mix in C-tube to inhibit the protease.
 - △ 3 mL ice-cold 10% FBS/PBS
- Transfer digest mix to a 15 mL conical. Spin 600 G for 5 minutes at 4 °C; discard supernatant; re-suspend cell pellet in 2 mL ice-cold PBS/BSA.
 - **\$** 4 °C
 - ♦ 00:05:00 600 g spin

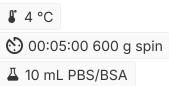


∆ 2 mL re-suspend in PBS/BSA

11 Filter re-suspended cells using 30 uM filter on sterile 50 mL conical on ice - rinse filter with 4 mL ice-cold PBS/BSA. Transfer flow-through to 15 mL conical.

4 mL rinse filter

12 Spin 15 mL conical tube containing filtered cells 600 G for 5 minutes at 4 °C; discard supernatant and re-suspend pellet in 10 mL ice-cold PBS/BSA.



- 13 Repeat rinse/spin in ice-cold PBS/BSA.
- 14 Remove supernatant and re-suspend in 1-2 mL ice-cold PBS/BSA.
- 15 Examine using hemocytometer and adjust concentration to 100 cells/uL for DropSeq or 1,000 cells/µL for 10X Chromium.