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

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Andrew Potter[1], Steve Potter[1]
[1]CCHMC

Human Cell Atlas Metho...

Andrew Potter
CCHMC

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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 *Bacillus Licheniformis* (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	Supplier	Catalog no.
	gentleMACS dissociator	Miltenyi	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	*B. Lich* (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 µL 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.

3 Weigh out 25 mg of tissue for each tube of B. Lich. enzyme mix (2 tubes total).
25 mg

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
00:02:00

5 Monitor digestion by taking small aliquot and visualizing under scope (every 5 minutes).
00:05:00

6 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

7 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

8 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
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

10 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.

4 °C

00:05:00 600 g spin

10 mL 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.