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

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

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Human Cell Atlas Method Development Community

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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 expression 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 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	Supplier	Catalog no.
gentleMACS dissociator	Miltenyi	130-093-235

The protocol workflow is as follows:

PROTOCOL integer ID:
13253

Keywords: CAP, cold-active
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- 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:

	V o l u m e (μ l)	R e a g e n t	F i n a l c o n c e n t r a t i o n
	8 9 4	D P B S	1 X

1	0.5 M EDTA	0.5 M
5	DNAse 1 (250 U / 10 μ L)	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 μ L of 10% BSA stock.

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

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 μ L - 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/ μ L for 10X Chromium.