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P1 Kidney Cold-Active Protease Single Cell Dissociation V.3

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Andrew Potter[1], Steve Potter[1]

[1]CCHMC

Andrew Potter
CCHMC

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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

	A	B
	Reagent	**Storage Condition**
	DPBS (Thermofisher, 14190144)	4°C
	1 M CaCl2	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 (StemCell, 07469)	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:

	A	B	C
	Volume (µl)	**Reagent**	**Final concentration**
	890	DPBS	1X
	5	1 M CaCl2	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 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

6 After MACS, briefly quick spin the MACS tube (to 300 G) at 4 °C to ensure contents are in the bottom of the tube.

4 °C

7 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

8 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

9 Transfer digest mix to a 15 mL conical. Spin 300 G for 5 minutes at 4 °C; discard supernatant; re-suspend cell pellet in 2 mL ice-cold PBS/BSA. 5m

4 °C

00:05:00 300 g spin

2 mL re-suspend in PBS/BSA

10 Filter re-suspended cells using 30 uM filter on sterile 15 mL conical on ice - rinse filter with 8 mL ice-cold PBS/BSA.

8 mL rinse filter with PBS/BSA

11 Spin 15 mL conical tube containing filtered cells 300 G for 5 minutes at 4 °C; discard supernatant and re-suspend pellet in 10 mL ice-cold PBS/BSA. 5m

4 °C

00:05:00 300 g spin

10 mL PBS/BSA

12 Repeat rinse/spin in ice-cold PBS/BSA.

13 Remove supernatant and re-suspend in 1-2 mL ice-cold PBS/BSA.

14 Examine using hemocytometer and adjust concentration to 100 cells/uL for DropSeq or 1,000 cells/µL for 10X Chromium.