P1 Kidney Cold-Active Protease Single Cell Dissociation

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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.

Citation: Andrew Potter,Steve Potter P1 Kidney Cold-Active Protease Single Cell Dissociation. **protocols.io** dx.doi.org/10.17504/protocols.io.nzkdf4w
Published: 23 Mar 2018

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.

Protocol

Step 1.
Extract & isolate P1 kidneys in ice-cold PBS.

Step 2.
Mince kidneys on top of petri dish, on ice, using razor blade.

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

AMOUNT
25 mg Additional info:

Step 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.

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

Step 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.

TEMPERATURE
4 °C Additional info:

Step 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.

TEMPERATURE
4 °C Additional info:

Step 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.

Step 9.

Add 3 mL ice-cold 10% FBS/PBS to digest mix in C-tube to inhibit the protease.

AMOUNT
3 ml Additional info: ice-cold 10% FBS/PBS

Step 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.

AMOUNT
2 ml Additional info: re-suspend in PBS/BSA

TEMPERATURE
4 °C Additional info:

Step 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.

AMOUNT
4 ml Additional info: rinse filter

Step 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.

AMOUNT
10 ml Additional info: PBS/BSA
TEMPERATURE
4 °C Additional info:

Step 13.

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

Step 14.

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

Step 15.
Examine using hemocytometer and adjust concentration to 100 cells/uL for DropSeq or 1,000 cells/µL for 10X Chromium.