Adult mouse kidney dissociation (on ice) V.9

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Protocol for adult (8-10 week) mouse kidney dissociation performed on ice to reduce artifact gene expression. The protocol is based on our protocol published in *Development* for P1 mouse kidney, however this protocol includes two layers to provide additional enzymatic digestion. Cell viability is ~80% with major kidney cell types represented.

Adult mouse kidney cell isolation Protocol.docx

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***Bacillus Licheniformis* Enzyme Mix (2 x 1 mL per 25 mg tissue):**
100 µL b. lich 100 mg/mL (10 mg/mL final) (Sigma, P5380)
5 µL 1 M CaCl2 (5 mM)
5 µL DNAse (125 U) (StemCell, #07469)
890 µL DPBS (no Ca, Mg) ThermoFisher (cat. #14190)

Preparing enzymes:
The enzymes are made up in DPBS (#14190). They are aliquoted and stored at -80 ºC. FBS (for making the FBS/PBS) is heat-inactivated and sterile-filtered.

Required reagents:
Red Blood Cell Lysis Buffer - Sigma (R7757)

Required Equipment & Consumables:
Thermomixer
Centrifuges for 1.5 mL and 15 mL conicals (MLS)
Pipettes and pipet tips (MLS)
15 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filter (MLS)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice (MLS)
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

The protocol workflow is as follows:
A. Isolate Kidney
B. First layer
C. Second layer
D. Preparing cells for single cell analysis

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MATERIALS

DPBS (no Ca, no Mg) **Thermofisher Catalog #14190144**

RBC Lysis Buffer **Sigma Catalog #R7757**

-Prepare enzyme mixes and leave on ice.
-Cool centrifuges to 4 °C.
-Isolate and transport tissue in ice-cold DPBS.

Isolate kidney

1 Perfuse kidneys to remove RBC. Extract and isolate kidneys in ice-cold PBS. Leave kidneys in ice-cold PBS until ready to dissociate (Up to 1 hr).

2 Coarsely mince tissue in PBS.

00:02:00 mince on ice

Layer 1

3 Weigh out 25 mg coarsely minced tissue for each set of kidneys (remove PBS before weighing).

25 mg minced kidney tissue

4 Continue mincing kidneys on top of petri dish, on ice, using razor blade in small vol. (~50 µL) PBS. (1-2 min) until fine paste.

50 µL PBS

5 Prepare a separate 1 mL aliquot of B. Lich enzyme mix for each set of adult kidneys (prepare on ice). Use p200 w/cut tip to transfer minced kidney tissue from petri dish to tube of enzyme mix.

1 mL B. Lich enzyme mix

6 Incubate digest mix for 10 min on ice with trituration and shaking. Triturate 15 strokes using 1 mL pipet set to 600 µL every 2 min; shake vigorously every min. 13m

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00:01:00 shake vigorously

00:02:00 triturate 15X

00:10:00 incubate on ice

7 After 10 min, let tissue chunks settle on ice for 1 min. Save supernatant (700 µL, 70%) and apply to 30 µM filter on 15 mL conical. Rinse filter w/5 mL 10% FBS/PBS. Leave filter on 15 mL conical for the next layer. 1m

00:01:00 let tissue chunks settle

700 µL save supernatant

5 mL rinse filter with 5 mL 10% FBS/PBS

Layer 2

8 Add additional 700 µL B. Lich enzyme mix to residual tissue chunks. Continue incubating on ice 15-20 min with shaking and trituration, until tubules and glomeruli are fully broken up. 23m

700 µL B. lich enzyme mix

00:01:00 shake vigorously

00:02:00 triturate 15X

00:20:00 incubate on ice

9 Once digestion is adequate (tubules / glomeruli are broken up), triturate and add entire digest mix to same 30 µM filter as used in previous step on 15 mL conical.

10 Spin 15 mL conical with combined flow-through from layer 1 and layer 2 (isolated cells) 300 g for 5 min at 4 °C. 5m

00:05:00 300 g at 4 °C

Prepare cells for 10X Chromium / scRNA-Seq

11 Discard supernatant. If necessary, perform RBC removal according to manufacturers instructions.

12 Re-suspend cells in 10 mL 10% FBS/PBS. Spin 300 g for 5 min at 4 °C.

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10 mL 10% FBS/PBS

00:05:00 spin 300 g

13 Discard supernatant. Re-suspend cells in 500 µL 10% FBS/PBS (or other compatible buffer). Analyze cell viability and concentration using trypan blue dye exclusion with a hemocytometer. Adjust cell concentration to 700-1,200 cells/µL for 10X single cell 3'v3.1.

500 µL 10% FBS/PBS

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