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Adult mouse kidney dissociation (on ice) V.8

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

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Abstract

Protocol for adult (8-10 week) mouse kidney dissociation performed on ice to reduce artifact gene expression. The first layer, consisting of collagenase digestion, breaks down the tissue and releases some cells and glomeruli and tubules. The second layer consists of bacillus licheniformis digestion for 15 min. augmented with a thermomixer at 1400 RPM and passaging with a 27 gauge needle. The second layer is meant to thoroughly break up remaining tubules and glomeruli, releasing cells such as podocytes. The final yield is 250K cells from 18 mg tissue with 98% viability, approximately 14,000 cells/mg tissue. Approximately 1% of released cells are podocytes (visualized using kidneys from MAFB-GFP+ mice using a hemocytometer).

Attachments

PDF

Adult Mouse Kidney D...

47KB

Guidelines

Collagenase Enzyme Mix (1 mL per 18 mg tissue):
50 µL Coll. A 100 mg/mL (5 mg/mL final) Sigma (cat. #10103586001)
50 µL Coll. Type 4 100 mg/mL (5 mg/mL final) Worthington (cat. #LS004186)
125 U DNAse (5 µL) AppliChem (cat. #A3778)
5 mM CacI2 (5 µL of 1 M CaCl2)
890 µL DPBS (no Ca, Mg) Thermo Fisher (cat. #14190)

***Bacillus Licheniformis* Enzyme Mix (1 mL per 18 mg tissue):**
100 µL b. lich 100 mg/mL (10 mg/mL final) (Sigma, P5380)
1 µL 0.5 M EDTA (Sigma, A8806)
899 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.
Collagenase A,Collagenase 4 and *bacillus licheniformis*: 100 mg/mL in 100 µL aliquots.
DNAse: 250 U/10 µL in 20 µL aliquots.

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

Optional Dead Cell Removal Kit:
EasySep dead cell removal (Annexin V) kit (cat. #17899)
EasySep Magnet (cat. #18000)

Required Equipment & Consumables:
Thermomixer
Centrifuges for 1.5 mL and 15 mL conicals (MLS)
Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
40 µM filters (MLS)
100 µM filters (MLS)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice (MLS)
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)
27 g x 1/2 needle w/syringe (BD, #309623)

The protocol workflow is as follows:
A. Isolate Kidney

B. First layer
C. Second layer
D. Preparing cells for Chromium

Materials

MATERIALS

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

RBC Lysis Buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7757

DNAse **AppliChem Catalog #**A3778

Before start

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

Isolate kidney

1 Transport kidney in ice-cold PBS.

2 Using razor blade, mince biopsy for ~2 min until fine paste on petri dish on ice.

00:02:00 mince on ice

Layer 1

3 Weigh out 18 mg of minced kidney onto petri dish. Transfer to 1.5 mL tube with 1 mL of collagenase enzyme mix on ice.

18 mg minced kidney tissue

4 Incubate for 20 min on ice. Shake tube every 30 sec. Triturate 10x every min (starting at 2 min), using p1000 set to 700 µL; with the first trituation cut off the end of the pipet tip with razor blade.

00:00:30 shake vigorously

00:01:00 triturate 10x

00:20:00 incubate on ice

5 After 20 min, let tissue chunks settle on ice 1 min. Spin tube 70 g for 30 sec at 4 ºC to spin down glomeruli and tubules.

00:01:00 settle one minute

00:00:30 spin 70 g

6 Remove 60% of supernatant (consisting of released cells) and apply to 40 µM filter on 50 mL conical. Rinse filter with 6 mL ice-cold PBS/BSA 0.04%.

600 µL save released cells in supernatant

6 mL ice-cold PBS/BSA

Layer 2

7 Add 1 mL PBS to tube containing remaining enzyme mix with tissue chunks. Triturate 10X. Apply to 100 µM filter on 50 mL conical. Rinse filter w/10 mL PBS. This step removes residual tissue chunks while saving glomeruli and tubules in the flow-through.

1 mL add 1 mL PBS

10 mL Rinse filter with 10 mL PBS

8 Transfer flow-through to 15 mL conical. Spin 300 g for five minutes to pellet flow-through, consisting of glomeruli and tubules.

9 Remove supernatant. Add 1 mL b. lich enzyme mix to tube containing the flow-through from the 100 µM filter (should be enriched in tubules and glomeruli). Triturate 10x. Transfer to 1.5 mL tube.

1 mL b. lich enzyme mix

10 For 15 min additional time (35 min total digestion) shake in thermomixer at 1400 RPM at 4 °C. Halfway through, stop and passage 5X w/27 gauge needle to help break up tubules and glomeruli.

00:15:00 shake in thermomixer

11 Triturate digest mix 10X and transfer to the same 40 µM filter on 50 mL conical. Rinse w/6 mL ice-cold PBS/BSA 0.04%.

6 mL ice-cold PBS/BSA

12 Transfer flow-through from previous step into a 15 mL conical.

13 Spin the tube with released cells 300 g for 5 min at 4 °C. Remove supernatant (leave ~100 µL).

00:05:00 spin 300 g

RBC Lysis

14 Add 900 µL of RBC lysis buffer to the 15 mL conical containing the cells. Triturate 10X using 1 mL pipet. Let sit two min on ice.

900 µL RBC lysis buffer

00:02:00 sit 2 min on ice

15 Add 10 mL ice-cold PBS/BSA 0.04% to dilute RBC lysis buffer.

10 mL ice-cold PBS/BSA

16 Spin 300 g for 5 min at 4 °C.

00:05:00 spin 300 g

Preparing cells for scRNA-Seq

17 Remove supernatant and re-suspend in 100-200 µL ice-cold PBS/BSA 0.04%. Check viability and concentration using hemocytometer with trypan blue.

100 µL ice-cold PBS/BSA

18 Optional: to increase the % of viable cells, at this point in the procedure you can perform dead cell removal using the EasySep dead cell removal kit according to the manufacturer's instructions.

19 Adjust concentration to 100 cells/µL for DropSeq or 1,000 cells/µL for Chromium.