

Adult mouse kidney dissociation (on ice) Version 5

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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).

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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 CaCl₂ (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)

1 µL 0.5 M EDTA

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 °C.

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


Before start

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

Materials

 DPBS (no Ca, no Mg) 14190144 by
ThermoFisher

 RBC Lysis Buffer R7757 by Sigma

 DNase A3778 by AppliChem

Protocol

Step 1.

Transport kidney in ice-cold PBS.

Step 2.

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

Step 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.

 AMOUNT

18 mg Additional info:
minced kidney tissue

Step 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 μ L; with the first trituration cut off the end of the pipet tip with razor blade.

Step 5.

After 20 min, let tissue chunks settle on ice 1 min. Spin tube 70 g for 30 sec at 4 $^{\circ}$ C to spin down glomeruli and tubules.

Step 6.

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

 AMOUNT

600 μ L Additional info:
save released cells in
supernatant

 AMOUNT

6 ml Additional info: ice-
cold PBS/BSA

Step 7.

Add 1 mL PBS to tube containing remaining enzyme mix with tissue chunks. Triturate 10X. Apply to 100 μ M 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.

 AMOUNT

1 ml Additional info: add 1
mL PBS

 AMOUNT

10 ml Additional info:
Rinse filter with 10 mL
PBS

Step 8.

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

Step 9.

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

 AMOUNT

1 ml Additional info: b. lich
enzyme mix

Step 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.

Step 11.

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

 AMOUNT

6 ml Additional info: ice-
cold PBS/BSA

Step 12.

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

Step 13.

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

 AMOUNT

900 μ L Additional info: RBC
lysis buffer

Step 14.

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

 AMOUNT

10 ml Additional info: ice-
cold PBS/BSA

Step 15.

Spin 300 g for 5 min at 4 °C.

Step 16.

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

 AMOUNT

100 μ L Additional info: ice-
cold PBS/BSA

Step 17.

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.

Step 18.

Adjust concentration to 100 cells/ μ L for DropSeq or 1,000 cells/ μ L for Chromium.
