Adult mouse kidney dissociation (on ice) Version 4

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Abstract
Protocol for adult (8-10 week) mouse kidney dissociation performed on ice to reduce artifact gene expression. The final yield (post dead-cell removal) is 90K cells from 13 mg tissue with 91% viability, approximately 7,000 cells/mg tissue. Prior to dead cell removal the yield is 110K cells from 13 mg tissue with 81% viability.

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Guidelines

Collagenase Enzyme Mix (2 x 1 mL):

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)
100 µg/mL soybean trypsin inhibitor (100 µL of 1 mg/mL) Sigma (cat. #10109886001)
125 U DNAse (5 µL) AppliChem (cat. #A3778)
5 mM Cacl2 (5 µL of 1 M CaCl2)
790 µL DPBS (no Ca, Mg) Thermo Fisher (cat. #14190)

Preparing enzymes and trypsin inhibitor:

The enzymes and soybean trypsin inhibitor are made up in DPBS (#14190). They are aliquoted and stored at -80 ºC.
Collagenase A and Collagenase 4: 100 mg/mL in 100 µL aliquots.
Soybean trypsin inhibitor: 1 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:

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)
30 µM filters - Miltenyi (130-098-458)
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 Chromium

Before start

-Prepare two one mL tubes of collagenase mix per 13 mg of tissue (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 DPBS.

Step 2.
Using razor blade, mince biopsy into 1-mm3 pieces on petri dish on ice for 2 min until fine paste.

Step 3.
Weigh out 13 mg of minced kidney onto petri dish. Transfer to 1.5 mL tube with 1 mL of enzyme mix on ice.

AMOUNT

13 mg Additional info:

Step 4.
Incubate on ice for 25 min. Shake tube every min. Triturate 10x every 3 min (starting at 2 min), using

p1000 set to 700 µL with the end of the tip cut off.

Step 5.
After 25 min, let tissue chunks settle on ice 1 min. Remove 80% of supernatant (consisting of released cells) and apply to 30 µM filter on 50 mL conical. Rinse filter with 5 mL ice-cold PBS/BSA 0.04%.

AMOUNT

5 ml Additional info: ice-cold PBS/BSA 0.04%

Step 6.
Transfer flow-through to 15 mL conical. Add additional 5 mL ice-cold PBS/BSA 0.04% to flow-through.

AMOUNT

5 ml Additional info: ice-cold PBS/BSA 0.04%

Step 7.
Spin flow-through 650 g for 5 min at 4 °C. Remove supernatant. Re-suspend cells in 10 mL ice-cold PBS/BSA 0.04% and leave on ice while continuing with the next layer.

AMOUNT

10 ml Additional info: ice-cold PBS/BSA 0.04%

TEMPERATURE

4 °C Additional info:

Step 8.
Add additional 1 mL enzyme mix to tube containing tissue chunks. Continue triturating 10x every 3 min and shaking every min while incubating on ice for an additional 25 min.

AMOUNT

1 ml Additional info: enzyme mix

Step 9.
After 25 min additional time (50 min total) triturate 10x and transfer entire volume of digest mix to a new 30 µM filter on a 50 mL conical tube. Rinse filter with 5 mL ice-cold PBS/BSA 0.04%.

AMOUNT

5 ml Additional info: ice-cold PBS/BSA 0.04%

Step 10.
Transfer flow-through to 15 mL conical. Bring volume to 10 mL with ice-cold PBS/BSA 0.04%.

AMOUNT

10 ml Additional info: ice-cold PBS/BSA 0.04%

Step 11.
Spin this tube and the tube from previous layer (two 15 mL conicals) 650 g for 5 min at 4 °C. Remove supernatant.

TEMPERATURE

4 °C Additional info:

Step 12.
Add 1 mL of RBC lysis buffer (room temp) to the tubes and combine to one 15 mL conical. Triturate 20X using 1 mL pipet. Let sit three min on ice. Add 10 mL ice-cold PBS/BSA 0.04%.

AMOUNT

1 ml Additional info: RBC lysis buffer

AMOUNT

10 ml Additional info: ice-cold PBS/BSA 0.04%

Step 13.
Spin 650 g for 5 min at 4 °C. Remove supernatant and re-suspend in 100-200 µL ice-cold PBS/BSA 0.04%. Check viability and concentration using hemocytometer with trypan blue.

AMOUNT

100 µl Additional info: re-suspend in ice-cold PBS/BSA 0.04%

TEMPERATURE

4 °C Additional info:

Step 14.
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 15.
Adjust concentration to 100 cells/µL for DropSeq or 1,000 cells/µL for Chromium.