Adult mouse kidney dissociation Version 2

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Abstract
Protocol for adult (8-10 week) mouse kidney dissociation.

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Guidelines

Storage Conditions of Reagents

Reagent	Storage Condition
DPBS (no Ca, no Mg)	4°C
0.5 M EDTA	room temp.
RBC Lysis Buffer	4°C
Protease from *Bacillus Licheniformis*	Store 100 µL aliquots (100 mg/mL) in DPBS at -80°C
DNAse	Store 10 µL aliquots (250 U/10 µL) in DPBS at -80°C

Required Equipment

Equipment	Supplier	Catalog no.
Thermomixer C or R	Eppendorf	5382000015 / Z605271

The protocol workflow is as follows:

A. Isolate Kidney
B. First layer
C. Second layer
D. Third layer
F. Preparing cells for Chromium

Before start

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

Materials

DPBS (no Ca, no Mg) 14190144 by Thermofisher

0.5 M EDTA AM9260G by Ambion

RBC Lysis Buffer R7757 by Sigma

Protease from Bacillus Licheniformis P5380 by Sigma

DNAse A3778 by AppliChem

Thermomixer C or R 5382000015 / Z605271 by Eppendorf

Protocol

Isolate Kidney

Step 1.

Quickly dissect and isolate kidney and transfer to ice-cold PBS.

Isolate Kidney

Step 2.

Remove fatty tissue and kidney capsule in ice-cold PBS.

Isolate Kidney

Step 3.

Mince whole kidney on petri dish, on ice for 2 min until fine.

Isolate Kidney

Step 4.
On petri dish weigh out 25 mg tissue. Using razor blade, transfer tissue to 1.5 mL tube containing 1 mL of enzyme mix (10 mg/mL b. lich).

First layer

Step 5.
Incubate tube on ice for 2 min. Shake every 30 seconds.

First layer

Step 6.
After 2 min total time, triturate gently 20x using 1 mL pipet set to 700 µL.

First layer

Step 7.
Triturate 10x every 2 minutes for 10 additional minutes (12 min total time) while incubating on ice.

First layer

Step 8.
Spin digest mix at 4° C 10 sec at 50 g to spin down cell clumps and leave dissociated cells in supernatant.

TEMPERATURE

4 °C Additional info:

First layer

Step 9.
Remove 80% of supernatant containing single cells and apply to 30 µM filter on 50 mL conical; rinse filter with 8 mL ice-cold PBS/BSA 0.04% into 50 mL conical. Save conical with filter for subsequent steps.

AMOUNT

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

Second layer

Step 10.
Add additional 1 mL enzyme mix (10 mg/mL b. lich) to residual tissue chunks.

AMOUNT

1 ml Additional info: b. lich
enzyme mix

Second layer

Step 11.
Triturate 10x with 1 mL pipet set to 700 µL.

Second layer

Step 12.

Continue digesting while shaking in thermomixer, set to 4 ºC at 1200 RPM for 12 additional min (24 min total). Every 4 min passage 8X with 18 gauge needle (3X total).

TEMPERATURE

4 °C Additional info: set thermomixer to 4 ºC (can leave in cold room)

Second layer

Step 13.

Spin at 4° C 10 sec for 50 g to spin down clumps of tissue, leaving released cells in supernatant.

TEMPERATURE

4 °C Additional info:

Second layer

Step 14.

Pipet 80% of supernatant containing released cells to the 30 µM filter (the same tube/filter as used in previous steps). Rinse filter with 8 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Third layer

Step 15.

Add additional 1 mL enzyme mix (10 mg/mL b. lich) to residual tissue chunks.

AMOUNT

1 ml Additional info: b. lich enzyme mix

Third layer

Step 16.

Continue dissociating remaining clumps at 1400 RPM in thermomixer at 4° C for 12 additional minutes (36 min total). Every 4 min passage 8X w/18 gauge needle w/1 mL syringe (3X total).

TEMPERATURE

4 °C Additional info:

Third layer

Step 17.

Triturate 10x and apply total volume of remaining digest mix to the same 30-µM filter used in previous steps.

Third layer

Step 18.

Rinse filter with 8 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Preparing cells for Chromium

Step 19.

Transfer flow-through to two 15 mL conicals.

Preparing cells for Chromium

Step 20.

Spin 500 G for 5 min at 4° C.

TEMPERATURE

4 °C Additional info: Spinning

Preparing cells for Chromium

Step 21.

Remove supernatant.

Preparing cells for Chromium

Step 22.

Re-suspend both tubes (combined) in 100 µL total volume PBS/BSA 0.04% and add 900 µL RBC lysis buffer (in 15 mL conical).

AMOUNT

100 µl Additional info: PBS/BSA 0.04%

AMOUNT

900 µl Additional info: RBC lysis buffer

Preparing cells for Chromium

Step 23.

Triturate 20x.

Preparing cells for Chromium

Step 24.

Let sit 2 min on ice.

Preparing cells for Chromium

Step 25.

Add additional 9 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Preparing cells for Chromium

Step 26.

Spin 500 G for 5 min at 4° C.

TEMPERATURE

4 °C Additional info: Spinning

Preparing cells for Chromium

Step 27.

Re-suspend pellet in 1 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Preparing cells for Chromium

Step 28.

Analyze using hemocytometer with trypan blue. Adjust concentration to 1000 cells / µL for 10x Chromium or 100 cells / µL for DropSeq.