Adult mouse kidney dissociation

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Abstract

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

Citation: Andrew Potter Adult mouse kidney dissociation. **protocols.io**
dx.doi.org/10.17504/protocols.io.nf9dbr6
Published: 08 Mar 2018

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 in ice-cold PBS.

Isolate Kidney

Step 2.

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

Isolate Kidney

Step 3.

Mince on petri dish, on ice (2 min) until fine.

Isolate Kidney

Step 4.

Weigh out 25 mg tissue per 1 mL enzyme mix (10 mg/mL).

First layer

Step 5.

Place tissue in eppendorf tube containing 1 mL digest mix on ice for 2 min.

First layer

Step 6.

Triturate gently 20x using 1 mL pipet set to 700 µL.

First layer

Step 7.

During the first 10 minutes, remove tube and shake every minute to re-suspend tissue chunks.

First layer

Step 8.

Triturate 10x every 2 minutes for 11.5 min.

First layer

Step 9.

Spin at 4° C 10 sec for 50 g to spin down cell clumps.

TEMPERATURE

4 °C Additional info: Spinning

First layer

Step 10.

Remove 80% of supernatant containing single cells and filter using 30 µM filter on 50 mL conical -

rinse with 8 mL PBS/BSA 0.01% into 50 mL conical. Save conical with filter for subsequent steps.

AMOUNT
8 ml Additional info: PBS/BSA

Second layer

Step 11.

Add additional 1 mL enzyme mix to residual tissue chunks.

AMOUNT
1 ml Additional info: Enzyme mix

Second layer

Step 12.

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

Second layer

Step 13.

Continue digesting while shaking in thermomixer at 1200 RPM for 23.5 min. Every 3 min passage 8X with 18 gauge needle (3X total).

Second layer

Step 14.

Spin at 4° C 10 sec for 50 g to spin down cell clumps.

TEMPERATURE
4 °C Additional info: Spinning

Second layer

Step 15.

Pipet 80% of supernatant and add to 30 µM filter. Rinse with 8 mL PBS/BSA into the same 50 mL conical as first digestion.

AMOUNT
8 ml Additional info: PBS/BSA

Third layer

Step 16.

Add additional 1 mL enzyme mix to residual tissue chunks.

AMOUNT
1 ml Additional info: Enzyme mix

Third layer

Step 17.

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

TEMPERATURE
4 °C Additional info: Digesting

Third layer

Step 18.

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

Third layer

Step 19.

Rinse filter with 8 mL PBS/BSA.

AMOUNT
8 ml Additional info: PBS/BSA

Preparing cells for Chromium

Step 20.
Transfer flow-through to two 15 mL conicals.

Preparing cells for Chromium

Step 21.

Spin 500 G for 5 min at 4° C.

TEMPERATURE
4 °C Additional info: Spinning

Preparing cells for Chromium

Step 22.

Remove supernatant.

Preparing cells for Chromium

Step 23.

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

AMOUNT
100 µl Additional info: PBS/BSA
AMOUNT
900 µl Additional info: RBC lysis buffer

Preparing cells for Chromium

Step 24.

Triturate 20x.

Preparing cells for Chromium

Step 25.

Let sit 2 min on ice.

Preparing cells for Chromium

Step 26.
Add additional 9 mL PBS/BSA.

AMOUNT

9 ml Additional info: PBS/BSA

Preparing cells for Chromium

Step 27.

Spin 500 G for 5 min at 4° C.

TEMPERATURE

4 °C Additional info: Spinning

Preparing cells for Chromium

Step 28.

Re-suspend pellet in 1 mL PBS/BSA.

AMOUNT

1 ml Additional info: PBS/BSA

Preparing cells for Chromium

Step 29.

Analyze using hemocytometer with trypan blue.