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Version 2

Adult mouse kidney dissociation V.2

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Human Cell Atlas Metho...



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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.

Attachments



[kidney_12_19_17.pdf](#)

154KB

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 <i>Bacillus Licheniformis</i>	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

Materials

MATERIALS

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

 0.5 M EDTA **Ambion Catalog #AM9260G**

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

 Protease from *Bacillus Licheniformis* **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5380**

 DNase **AppliChem Catalog #A3778**

 Thermomixer C or R **Eppendorf Catalog #5382000015 / Z605271**

Troubleshooting

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	<i>B. Lich</i> (100 mg/mL)	10 mg/mL







+25 mg tissue / 1 mL enzyme mix



Isolate Kidney

- 1 Quickly dissect and isolate kidney and transfer to ice-cold PBS.
- 2 Remove fatty tissue and kidney capsule in ice-cold PBS.
- 3 Mince whole kidney on petri dish, on ice for 2 min until fine.
 00:02:00 Mincing
- 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

- 5 Incubate tube on ice for 2 min. Shake every 30 seconds.
 00:02:00 Incubation on ice
 00:00:30 shake every 30 sec
- 6 After 2 min total time, triturate gently 20x using 1 mL pipet set to 700 μ L.
- 7 Triturate 10x every 2 minutes for 10 additional minutes (12 min total time) while incubating on ice.
 00:10:00 incubate on ice
 00:02:00 Triturate 10x
- 8 Spin digest mix at 4° C 10 sec at 50 g to spin down cell clumps and leave dissociated cells in supernatant.
 4 °C
 00:00:10 spin 50 g for 10 sec.
- 9 Remove 80% of supernatant containing single cells and apply to 30 μ M 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.



🧪 8 mL ice-cold PBS/BSA 0.04%

Second layer

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

🧪 1 mL b. lich enzyme mix

- 11 Triturate 10x with 1 mL pipet set to 700 μ L.

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

⌚ 00:12:00 digest in thermomixer at 1200 RPM

⌚ 00:04:00 every 4 min passage 8X w/18 gauge needle (3X total)

🌡 4 °C set thermomixer to 4 °C (can leave in cold room)

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

🌡 4 °C

⌚ 00:00:10 Spin at 50 g for 10 sec.

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

🧪 8 mL ice-cold PBS/BSA 0.04%

Third layer

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

🧪 1 mL b. lich enzyme mix

- 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).

🌡 4 °C


⌚ 00:12:00 thermomixer at 1400 RPM

⌚ 00:04:00 every 4 min passage 8X with 18 gauge needle

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



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


 8 mL ice-cold PBS/BSA 0.04%

Preparing cells for Chromium

19 Transfer flow-through to two 15 mL conicals.


20 Spin 500 G for 5 min at 4° C.

 4 °C Spinning

 00:05:00 Spin at 500 g

21 Remove supernatant.


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).

 100 µL PBS/BSA 0.04%

 900 µL RBC lysis buffer

23 Triturate 20x.

24 Let sit 2 min on ice.


 00:02:00 On ice

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


 9 mL ice-cold PBS/BSA 0.04%

26 Spin 500 G for 5 min at 4° C.

 4 °C Spinning

 00:05:00 Spin at 500 g

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

 1 mL ice-cold PBS/BSA 0.04%

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

