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Adult human kidney tissue cell dissociation (on ice)

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Andrew Potter[1]

[1]University of Cincinnati

Human Cell Atlas Metho...

Andrew Potter
CCHMC

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol can be used to dissociate adult human kidney “on ice” - maintaining authentic gene expression profiles. It was designed using a mix of Collagenases (Type 4, and A) which provide broad proteolytic activity, but preferentially cleave extracellular bonds, largely leaving cells intact. The total incubation time is 1 hour 20 minutes divided into two layers. At the end of the procedure, RBC lysis is performed. The total yield at the end of the procedure is ~1200 (non-RBC) cells released per mg tissue with 87% viability.

In the digest mix, there is trypsin inhibitor from soybean which is designed to limit the activity of tryptic proteins in the collagenase mix which can damage the integrity of the cell. There is also 5 mM CaCl2, an activator of collagenase activity, in addition to DNAse - which chews up DNA released from dead cells, reducing cell clumping. The dissociation itself it carried out in two layers. The first layer is 30 minutes and includes trituration and shaking. After this layer, tissue clumps are settled for 1 min, and the supernatant containing released cells is removed and filtered using a 30 µM filter and rinsed with ice-cold PBS-BSA. This helps to preserve the integrity of released cells while continuing the digest clumps of undissociated cells. To the residual clumps, an additional 1 mL of enzyme mix is added and the digestion is continued for 50 additional minutes (1 hr 20 mins total time).

Guidelines

Collagenase Enzyme Mix: 2 × 1 mL (make two tubes)
75 µL Collagenase Type A 100 mg/mL (7.5 mg/mL final concentration)
75 µL Collagenase Type 4 100 mg/mL (7.5 mg/mL final)
100 µL of soybean trypsin inhibitor 1 mg/mL (100 µg/mL final)
5 µL DNAse (125 U/mL final)
5 µL of 1 M CaCl2 (5 mM final)
740 µL DPBS (no Ca, no Mg)

Reagents
Enzymes, trypsin inhibitor, BSA and DNAse are made up in DPBS (no Ca, no Mg) from Thermo Fisher (14190).
Bovine Serum Albumin - Sigma (A8806).
DNAse - Applichem (A3778) - 10 µL aliquots in PBS each with 250 U.
soybean trypsin inhibitor - Roche (10109886001) - 100 µL aliquots of 1 mg/mL.
Collagenase A - Roche (10103586001) - 100 µL aliquots of 100 mg/mL - frozen at -80 °C.
Collagenase Type 4 - Worthington (LS004186) - 100 µL aliquots of 100 mg/mL - frozen at -80 °C
Red Blood Cell Lysis Buffer - Sigma (R7757)
Trypan Blue Solution 0.4% - Gibco (15250061)

Equipment
Centrifuge for 1.5 mL, 15 mL conicals
Pipettes and pipet tips
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
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Troubleshooting

1 Transport kidney in ice-cold PBS.

2 Mince biopsy into 1-mm3 pieces using razor blade on petri dish on ice.

3 Weigh out 10 mg of minced kidney onto petri dish. Transfer to 1.5 mL tube with 1 mL of enzyme mix on ice.

4 Shake tube every 1 min. Triturate 10x every 3 min (starting at 2 min), using p1000 set to 700 µL with the end of the tip cut off.
00:03:00 triturate 10X
00:01:00 shake

5 After 30 min of incubating on ice, let tissue chunks settle on ice 1 min.
00:30:00 incubate on ice

6 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%
5 mL ice-cold PBS/BSA 0.04%

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

8 Spin flow-through 650 g for 5 min at 4 °C. Remove supernatant. Re-suspend cells in 10 mL PBS/BSA 0.04% and leave on ice.
4 °C spin 650 g for 5 min
10 mL re-suspend cells in ice-cold PBS/BSA 0.04%

9 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.
1 mL add enzyme mix to tissue chunks
00:03:00 triturate 10X
00:01:00 shake

10 After 50 min additional time (1 hr. 20 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 w/5 mL ice-cold PBS/BSA 0.04%.
00:50:00 incubate on ice w/trituration

5 mL ice-cold PBS/BSA 0.04%

11 Transfer flow-through to 15 mL conical. Bring volume to 10 mL with ice-cold PBS/BSA 0.04%. Spin this tube and the tube from previous layer (two tubes) 650 g for 5 min at 4 °C. Remove supernatant.

10 mL ice-cold PBS/BSA 0.04%

4 °C spin 650 g for 5 min

12 Add 2 mL of RBC lysis buffer 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% to dilute RBC lysis buffer.

2 mL RBC lysis buffer

10 mL ice-cold PBS/BSA 0.04%

13 Spin 650 g for 5 min at 4 °C. Remove all but 100 µL of supernatant.

14 To 100 µL of cells, repeat RBC lysis: add 900 µL of RBC lysis buffer. Triturate 10x and let sit one min. on ice. Add 10 mL of ice-cold PBS/BSA 0.04%. Spin 650 g for 5 min at 4 ºC. Remove as much supernatant as possible.

900 µL RBC lysis buffer

10 mL ice-cold PBS/BSA 0.04%

4 °C Spin 650 g for 5 min

15 Re-suspend in ~100 µL ice-cold PBS/BSA 0.04%. Check viability and concentration using hemocytometer with trypan blue.