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Adult mouse pancreas cell dissociation (on ice) V.2

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dx.doi.org/10.17504/protocols.io.ewov1yj6kvr2/v2

Human Cell Atlas Method Development Community


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ABSTRACT

Note: In testing several different enzymes to dissociate pancreas tissue on ice, Collagenase Type 4 appeared to be most effective. However, the results of the dissociation have been variable, with significant debris in some preps, and cell type representation has not validated using 10X or flow; further optimization may be needed with this protocol.

This procedure is used to dissociate adult (10 wk.) mouse pancreas into single cells. The procedure is carried out on ice in order to maintain a more authentic gene expression profile. It is a two-layered dissociation, with each layer consisting of 5 mg/mL type 4 collagenase. The yield is 4400 cells/mg with 94% viability.

ATTACHMENTS

Pancreas Cell Dissociation.pdf

DOI

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PROTOCOL CITATION

Andrew Potter 2022. Adult mouse pancreas cell dissociation (on ice). **protocols.io** https://protocols.io/view/adult-mouse-pancreas-cell-dissociation-on-ice-cfi3tkgn Version created by Andrew Potter

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GUIDELINES

Type 4 Collagenase Enzyme Mix (2 x 1 mL)

50 µL Type 4 Collagenase 100 mg/mL (5 mg/mL final conc.)
5 µL DNAse (125 U)
5 µL 1 M $CaCl_2$ (5 mM final conc.)
10 µL 10% BSA/PBS (0.1% BSA final conc.)
930 µL DPBS

Reagents

Collagenase Type 4 - Worthington (LS004186) - 100 µL aliquots of 100 mg/mL, frozen at -80 °C
DNAse 1 - Applichem (A3778) – 10 µL aliquots each with 250 U, frozen at -80 °C
DPBS - ThermoFisher (cat. #14190)
Red Blood Cell Lysis Buffer - Sigma (R7757)
Trypan Blue Solution 0.4% - Gibco (15250061)

Required Equipment & Consumables:

Refrigerated centrifuge
Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters (MACS SmartStrainers, 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:

1. Isolate pancreas
2. First layer
3. Second layer
4. Preparing cells for Chromium/DropSeq

BEFORE STARTING

-Prepare enzyme mixes and leave on ice.
-Cool centrifuges to 4 °C.
-Isolate and transport tissue in ice-cold DPBS.

Dissect pancreas and place in ice-cold PBS.

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2 Mince tissue thoroughly on petri dish on ice (2 min) until fine paste.
00:02:00 mince on ice

3 Weigh out 18 mg tissue and add to tube with 1 mL Type 4 collagenase enzyme mix.
18 mg minced pancreas tissue

4 Incubate on ice. Shake vigorously every 30 seconds for the first two min to re-suspend tissue.
00:00:30 shake vigorously for first 2 min

5 At two mins, begin triturating 10x every min. Continue triturating on ice for 20 min.
00:20:00 triturate on ice

6 After incubating 20 min, let chunks settle for 1 min on ice.
00:01:00 let chunks settle

7 Pipet top 75% (750 µL) of supernatant containing released cells onto a 30 µM filter on a 50 mL conical, on ice.
750 µL save supernatant

8 Rinse filter with 5 mL ice-cold PBS/BSA 0.04%. Save filter and flow-through for next steps.
5 mL ice-cold PBS/BSA 0.04%

9 To residual tissue chunks add additional 1 mL type 4 collagenase enzyme mix.
1 mL type 4 collagenase mix

10 Continue triturating on ice 10x every min for 30 additional min (50 min total digest time).
00:30:00 triturate on ice

11 Triturate and add entire volume to same 30 µM filter on 50 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%.
5 mL ice-cold PBS/BSA 0.04%

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12 Transfer flow-through to 15 mL conical. Spin 300 g for 5 min at 4 °C.
00:05:00 spin 300 g at 4 °C

13 Remove supernatant and re-suspend in 100 µL ice-cold PBS/BSA 0.04%.

14 Add 900 µL RBC lysis buffer. Triturate 20x and incubate 2 min. on ice.
00:02:00 incubate on ice

15 Add 10 mL ice-cold PBS/BSA 0.04% to dilute RBC lysis buffer.

16 Spin 300 g for 5 min at 4 °C. Remove supernatant.
00:05:00 spin 300 g

17 Re-suspend in 100 µL ice-cold PBS/BSA 0.04%. Analyze viability and yield using a hemocytometer with trypan blue. Adjust concentration to 1,000 cells/µL for Chromium or 1,000 cells/µL for DropSeq.
100 µL ice-cold PBS/BSA 0.04%

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