

Aug 19, 2022

Version 2

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

DOI

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

Andrew Potter¹

¹CCHMC

Human Cell Atlas Metho...



Andrew Potter

CCHMC

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.ewov1yj6kvr2/v2>

Protocol Citation: Andrew Potter 2022. Adult mouse pancreas cell dissociation (on ice). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.ewov1yj6kvr2/v2> Version created by **Andrew Potter**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: In development

We are still developing and optimizing this protocol

Created: August 19, 2022

Last Modified: August 19, 2022

Protocol Integer ID: 68923

Keywords: CAP, pancreas, dissociation, single cell, adult mouse pancreas cell dissociation, mouse pancreas into single cell, pancreas tissue on ice, pancreas tissue, mouse pancrea, collagenase type, collagenase, cell, gene expression, several different enzyme, layered dissociation, cell type representation, results of the dissociation

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 Dissoc...

47KB



Guidelines

Type 4 Collagenase Enzyme Mix (2 × 1 mL)

50 µL Type 4 Collagenase 100 mg/mL (5 mg/mL final conc.)

5 µL DNase (125 U)

5 µL 1 M CaCl₂ (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












Troubleshooting



Before start

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



- 1 Dissect pancreas and place in ice-cold PBS.
- 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 μ L) of supernatant containing released cells onto a 30 μ M filter on a 50 mL conical, on ice.
 750 μ L 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 μ M filter on 50 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%.
 5 mL ice-cold PBS/BSA 0.04%
- 12 Transfer flow-through to 15 mL conical. Spin 300 g for 5 min at 4 $^{\circ}$ C.
 00:05:00 spin 300 g at 4 $^{\circ}$ C
- 13 Remove supernatant and re-suspend in 100 μ L ice-cold PBS/BSA 0.04%.




14 Add 900 μ L 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 μ L ice-cold PBS/BSA 0.04%. Analyze viability and yield using a hemocytometer with trypan blue. Adjust concentration to 1,000 cells/ μ L for Chromium or 1,000 cells/ μ L for DropSeq.

 100 μ L ice-cold PBS/BSA 0.04%