Adult mouse pancreas cell dissociation (on ice)

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Abstract

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.

Citation: Andrew Potter Adult mouse pancreas cell dissociation (on ice). **protocols.io** dx.doi.org/10.17504/protocols.io.r8rd9v6
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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 start

-Prepare enzyme mixes and leave on ice.

-Cool centrifuges to 4 °C.

-Isolate and transport tissue in ice-cold DPBS.

Protocol

Step 1.
Dissect pancreas and place in ice-cold PBS.

Step 2.
Mince tissue thoroughly on petri dish on ice (2 min) until fine paste.

DURATION
00:02:00 : mince on ice

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

AMOUNT
18 mg : minced pancreas tissue

Step 4.

Incubate on ice. Shake vigorously every 30 seconds for the first two min to re-suspend tissue.

DURATION

00:00:30 : shake vigorously for first 2 min

Step 5.

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

DURATION

00:20:00 : triturate on ice

Step 6.

After incubating 20 min, let chunks settle for 1 min on ice.

DURATION

00:01:00 : let chunks settle

Step 7.

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

AMOUNT

750 µl : save supernatant

Step 8.

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

AMOUNT

5 ml : ice-cold PBS/BSA 0.04%

Step 9.

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

AMOUNT

1 ml : type 4 collagenase mix

Step 10.

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

DURATION

00:30:00 : triturate on ice

Step 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%.

AMOUNT

5 ml : ice-cold PBS/BSA 0.04%

Step 12.

Transfer flow-through to 15 mL conical. Spin 300 g for 5 min at 4 °C.

DURATION

00:05:00 : spin 300 g at 4 °C

Step 13.

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

Step 14.

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

DURATION

00:02:00 : incubate on ice

Step 15.

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

Step 16.

Spin 300 g for 5 min at 4 °C. Remove supernatant.

DURATION

00:05:00 : spin 300 g

Step 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.

AMOUNT

100 µl : ice-cold PBS/BSA 0.04%