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Preparation of single-cell suspension from mouse pancreas

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We use this protocol and it's working

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Abstract

Preparation of single-cell suspension from mouse pancreas

Guidelines

1. Finely dissect the pancreas into small fragments using a blade.
2. Subject fragments to digestion in a solution containing 1 mg/ml collagenase type V (Solarbio, C8170) in DMEM/F12 (Gibco, C11330500BT) for 5-10 minutes at 37 °C.
3. Halt the enzymatic reaction by diluting the collagenase solution with chilled (4 °C) PBS.
4. Obtain cell pellets through centrifugation at 300g for 5 minutes at 4 °C.
5. Further digest pellets with 1 ml TrypLE Express (Gibco, 12604013) at 37 °C.
6. Terminate the reaction by diluting the solution with chilled (4 °C) PBS, and collect cell pellets by centrifugation at 300g for 5 minutes at 4 °C.
7. Incubate the cell pellet with a 200 U/ml DNase I (Solarbio, D8070) solution in DMEM/F12. Terminate the reaction by diluting with chilled (4 °C) PBS, and collect cell pellets by centrifugation at 300g for 5 minutes at 4 °C.
8. Resuspend the cells in chilled sorting buffer (PBS pH 7.2 containing 0.5% (wt/vol) BSA and 2 mM EDTA) for subsequent FACS analysis.
9. For mice treated with caerulein, further stain the cells with APC-conjugated CD45 antibody to exclude immune cells.

Materials

Pancreas tissue; blade (for dissection); Collagenase type V (Solarbio, C8170) prepared at 1 mg/ml in DMEM/F12 (Gibco, C11330500BT); DMEM/F12 (Gibco, C11330500BT); TrypLE Express (Gibco, 12604013); DNase I (Solarbio, D8070) prepared at 200 U/ml in DMEM/F12; Phosphate-buffered saline (PBS), chilled (4 °C); Sorting buffer: PBS pH 7.2 containing 0.5% (wt/vol) BSA and 2 mM EDTA; BSA; EDTA; APC-conjugated CD45 antibody (for exclusion of immune cells); Centrifuge capable of 300g; Equipment for FACS analysis.



Safety warnings

! This protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Ethics statement

This protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Before start

Prepare chilled (4 °C) PBS. Prepare collagenase type V at 1 mg/ml in DMEM/F12. Prepare DNase I at 200 U/ml in DMEM/F12. Prepare sorting buffer: PBS pH 7.2 with 0.5% (wt/vol) BSA and 2 mM EDTA. Pre-chill centrifuge and keep reagents and tubes on ice or at 4 °C where indicated. Have APC-conjugated CD45 antibody available if excluding immune cells (e.g., in caerulein-treated mice).



- 1 Finely dissect the pancreas into small fragments using a blade.
- 2 Subject fragments to digestion in a solution containing 1 mg/ml collagenase type V (Solarbio, C8170) in DMEM/F12 (Gibco, C11330500BT) for 5–10 minutes at 37 °C.
- 3 Halt the enzymatic reaction by diluting the collagenase solution with chilled (4 °C) PBS.
- 4 Obtain cell pellets by centrifugation at 300g for 5 minutes at 4 °C.
- 5 Further digest the pellets with 1 ml TrypLE Express (Gibco, 12604013) at 37 °C.
- 6 Terminate the TrypLE reaction by diluting the solution with chilled (4 °C) PBS and collect cell pellets by centrifugation at 300g for 5 minutes at 4 °C.
- 7 Incubate the cell pellet with 200 U/ml DNase I (Solarbio, D8070) in DMEM/F12; terminate the reaction by diluting with chilled (4 °C) PBS and collect cell pellets by centrifugation at 300g for 5 minutes at 4 °C.
- 8 Resuspend the cells in chilled sorting buffer (PBS pH 7.2 containing 0.5% (wt/vol) BSA and 2 mM EDTA) for subsequent FACS analysis.
- 9 For mice treated with caerulein, further stain the cells with APC-conjugated CD45 antibody to exclude immune cells.