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KAPP-Sen TMC: Dissociation of Pancreatic Islets (recovered) Protocol

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ABSTRACT

The dispersed samples were shipped cold from PRODOLABS. Prior to scRNA-seq dispersed samples from brain dead donor's pancreatic islets were recovered and dissociated as follows.

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

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Abstract

1 The dispersed samples were shipped from **PRODOLABS**. Prior to scRNA-seq dispersed samples from brain dead donor's pancreatic islets were recovered and dissociated as follows.

Cell Dissociation with Accutase

2 NOTE: Before beginning cell dissociation coat all the materials (pipettes, tubes, etc.) with PIM-S001GMP media to prevent sticking.

1. Transfer cell suspension of pure islets to a new 50ml tube. Use additional media to rinse original container.
2. Centrifuge at room temperature 130g for 5 mins.
3. Aspirate the supernatant and add media to the appropriate concentration of 1,000 islets/1 ml.
   --> Ex: If receiving 7,000 islets, use 7 ml media.
4. To recover cells, add half of the islets to a coated flask and incubate at 37C overnight.
5. Coat pipette and add islets from flask to a 50ml tube. Wash flask with media to make sure all islets have been collected.
6. Centrifuge at 130 g, room temperature for 2 mins.
7. Aspirate media and resuspend in 4 ml accutase. Incubate at 37C for 8 mins, mixing with pipette every 2 mins. Check at 6 mins.
   -->1 mL accutase/1,000 islets
8. Add CMRL 1066 (Cat. 11530037) media to 25 ml then centrifuge at 230 g.
9. Aspirate supernatant and resuspend in 1.5 ml of CMRL
10. Filter through a 40 µm Flowmi.
11. Count cells using AO/PI (acridine orage/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.
12. Proceed to cell fixation.

Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

3 Cells were fixated prior to scRNAseq according to https://dx.doi.org/10.17504/protocols.io.[...]/v1