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KAPP-Sen TMC: Dissociation of Pancreatic Islets (non-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 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 cold from **PRODOLABS**.
Prior to scRNA-seq dispersed samples from brain dead donor's pancreatic islets were 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. Add half of the islets (recovery) to the coated flask and put to incubate at 37C.
5. To the other half of the islets (no recovery), add media to 10 ml and centrifuge then aspirate carefully.
6. Add accutase at a concentration of 1 ml/1,000 islets. Mix with pipette and incubate at 37C. Coat pipette tip and use to mix every 2 mins, checking at 6 mins.
7. Add CMRL 1066 (Cat. 11530037) to approximately 9 ml media/1 ml accutase then centrifuge at 230 g.
8. Aspirate supernatant and resuspend in 1.5 ml of CMRL
9. Filter through a 40 µm Flowmi.
10. Count cells using AO/PI (acridine orage/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.
11. 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