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

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We use this protocol and it's working

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Cellular Senescence Network (SenNet) Method Development Community

KAPP-Sen TM

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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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TMC

Cell Dissociation with Accutase

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Note

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

1.1 Transfer cell suspension of pure islets to a new 50ml tube. Use additional media to rinse original container.

1.2 Centrifuge at room temperature 130g for 5 mins.

1.3 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.

1.4 To recover cells, add half of the islets to a coated flask and incubate at 37°C overnight.

1.5 Coat pipette and add islets from flask to a 50ml tube. Wash flask with media to make sure all islets have been collected.

1.6 Centrifuge at 130 g, room temperature for 2 mins.

1.7 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

1.8 Add CMRL 1066 (Cat. 11530037) media to 25 ml then centrifuge at 230 g.

1.9 Aspirate supernatant and resuspend in 1.5 ml of CMRL

1.10 Filter through a 40 µm Flowmi.

1.11 Count cells using AO/PI (acridine orage/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.

1.12 Proceed to cell fixation.

Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

2 Cells were fixated prior to scRNAseq according to https://dx.doi.org/10.17504/protocols.io.x54v9py5zg3e/v1