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

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Cellular Senescence Net...

KAPP-Sen TMC



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**We use this protocol and it's working**

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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.

## Troubleshooting

## 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 Add half of the islets (recovery) to the coated flask and put to incubate at 37°C.
- 1.5 To the other half of the islets (no recovery), add media to 10 ml and centrifuge then aspirate carefully.
- 1.6 Add accutase at a concentration of 1 ml/1,000 islets. Mix with pipette and incubate at 37°C. Coat pipette tip and use to mix every 2 mins, checking at 6 mins.
- 1.7 Add CMRL 1066 (Cat. 11530037) to approximately 9 ml media/1 ml accutase then centrifuge at 230 g.
- 1.8 Aspirate supernatant and resuspend in 1.5 ml of CMRL
- 1.9 Filter through a 40 µm Flowmi.
- 1.10 Count cells using AO/PI (acridine orange/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.



1.11 Proceed to cell fixation.

## Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

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