SEP 27, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ewov1qnrkgr2/v1

Protocol Citation: Juliana Alcoforado Diniz, Jessica Garofalo, Dylan Baker, Paul Robson 2023. KAPP-Sen TMC: Dissociation of Pancreatic Islets (non-recovered). **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1qnrkgr2/v1

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

Created: Aug 01, 2023

Last Modified: Sep 27, 2023

PROTOCOL integer ID: 85793

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

Juliana Alcoforado Diniz[1], Jessica Garofalo[1], Dylan Baker[1], Paul Robson[1,2,3]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA;
[2]Department of Genetics and Genome Sciences, University of Connecticut School of Medicine, Farmington, CT, USA;
[3]Institute for Systems Genomics, University of Connecticut, Farmington, CT, USA

Cellular Senescence Network (SenNet) Method Development Community

KAPP-Sen TM

Ashley M Raynock
UConn Health, UConn Center on Aging

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

Keywords: SenNet KAPP-Sen
TMC

Cell Dissociation with Accutase

1

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 orage/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

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