Mar 25, 2021

Human and Mouse Islet Single-cell Dispersion for Patch-clamp and Imaging

Aliya F Spigelman[1], Austin B[2], Xiaoqing Dai[1], Amanda Gomes[2], Patrick E Macdonald[1]

[1]University of Alberta; [2]PEM

1 Works for me dx.doi.org/10.17504/protocols.io.spiedke

CIRTNR2FIC

Aliya Spigelman
University of Alberta

SUBMIT TO PLOS ONE

ABSTRACT

Detailed protocol for dispersing human and mouse islets into single cells for patch clamp and imaging experiments

DOI

dx.doi.org/10.17504/protocols.io.spiedke

PROTOCOL CITATION

Aliya F Spigelman, Austin B, Xiaoqing Dai, Amanda Gomes, Patrick E Macdonald 2021. Human and Mouse Islet Single-cell Dispersion for Patch-clamp and Imaging. **protocols.io** https://dx.doi.org/10.17504/protocols.io.spiedke

CREATED

Aug 16, 2018

LAST MODIFIED

Mar 25, 2021

PROTOCOL INTEGER ID

14794

MATERIALS TEXT

MATERIALS

Penicillin-Streptomycin **Gibco - Thermo Fisher Catalog #15140122**

FBS (Canadian Origin) **Gibco - Thermo Fischer Catalog #12483-020**

DMEM **Gibco - Thermo Fischer Catalog #11885**

Cell Dissociation Buffer enzyme-free Hanks Balanced Salt Solution **Gibco - Thermo Fisher Catalog #13150-16**

1 Transfer islets into 10cm petri dish (non tissue culture treated), adding human islet culture media if needed.

Human islet culture media:

500ml DMEM (5mM glucose)	Gibco 11885
50ml FBS Canadian Origin	Gibco 12483-020
5ml Pen/strep	Gibco 15140-122

2 Under a stereomicroscope, hand pick islets into a 2nd 35-mm non tissue culture treated dish with Human islet culture media.

The number of islets will depend on how many dishes are required. Our group uses 10 islets/dish as a maximum final density (ie 60 islets for 6 dishes).

3 Repeat this into a subsequent 35-mm petri dishes until islets reach close to 100% purity.

4 Pick islets into 15 mL falcon tube with as little media as possible.

5 Add 1ml of Gibco cell dissociation buffer (cat # **13150-016**).

1 mL Cell dissociation buffer

6 Incubate in 37°C water bath for 10 minutes.

37 °C

00:10:00

7 Pipette islets up and down with 1 ml pipette to produce single cells

8 Add 9 ml of human islet culture media.

9 mL Human islet culture media

9 Centrifuge @200 RCF for 3 minutes.

00:03:00

Citation: Aliya F Spigelman, Austin B, Xiaoqing Dai, Amanda Gomes, Patrick E Macdonald (03/25/2021). Human and Mouse Islet Single-cell Dispersion for Patch-clamp and Imaging. https://dx.doi.org/10.17504/protocols.io.spiedke

10 Aspirate supernatant and resuspend pelleted cells in human islet media (~200 ul/dish)

11 Plate ~200 ul drop of suspended cells in centre of each tissue culture treated 35 mm dish.

12 Culture @ 37°C in 5% CO_2 incubator

37 °C 5% CO2

13 After 4 hours add 2 mL of human islet culture media.

04:00:00

2 mL Human islet culture media

Citation: Aliya F Spigelman, Austin B, Xiaoqing Dai, Amanda Gomes, Patrick E Macdonald (03/25/2021). Human and Mouse Islet Single-cell Dispersion for Patch-clamp and Imaging. https://dx.doi.org/10.17504/protocols.io.spiedke