MAR 25, 2021

OPEN ACCESS

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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 16, 2018

Last Modified: Mar 25, 2021

PROTOCOL integer ID:
 14794

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

Aliya F Spigelman¹, Austin B², Xiaoqing Dai¹, Amanda Gomes², Patrick E Macdonald¹

¹University of Alberta; ²PEM

CIRTNR2FIC

Aliya F Spigelman
 University of Alberta

ABSTRACT

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

MATERIALS

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 DME M (5mM glucose)	Gibco 11885
50ml FBS Cana dian Origin	Gibco 12483 -020
5ml Pen/s trep	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
- 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% CO₂ 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