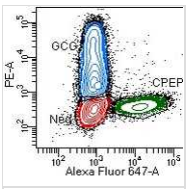

May 02, 2022

Measurement of pancreatic islet beta-cell proliferation by flow cytometry

Julien Ghislain[1], Vincent Poitout[2]

[1]Montreal Diabetes Research Center, CRCHUM, Montréal, QC, Canada.;
[2]Montreal Diabetes Research Center, CRCHUM, and Department of Medicine, Université de Montréal, Montréal, QC, Canada.

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Laboratory of Vincent Poitout
Tech. support email: **julien.ghislain.chum@ssss.gouv.qc.ca**

Julien Ghislain

This protocol describes the steps to assess pancreatic beta-cell proliferation by flow cytometry. It is suitable for islets isolated from both rodents and humans. We routinely apply this protocol to quantify total and proliferating beta-cells but, with the appropriate antibody-fluorophore conjugates, can be easily adapted to assess other islet cells types (eg. alpha and delta) and markers (eg. apoptosis). Briefly, islets labelled with the proliferation marker EdU are dispersed, dead cells labeled and fixed. EdU is detected using the Click-iT Plus EdU Flow Cytometry Assay Kit and beta-cells immunostained with fluorophore-coupled anti-insulin antibodies. Beta-cell proliferation is then determined by flow cytometry by calculating the percentage of double-positive cells for EdU and insulin over the corresponding total insulin-positive cell population.

DOI

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protocol

Pronounced proliferation of non-beta cells in response to beta-cell mitogens in isolated human islets of Langerhans. Maachi H, Ghislain J, Tremblay C, Poitout V. Sci Rep. 2021 May 28;11(1):11283. doi: 10.1038/s41598-021-90643-3. PMID: 34050242.

flow cytometry, pancreatic islet, beta-cell, proliferation

protocol ,

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Phosphate Buffered Saline 10x (solution) **Bio Basic Inc. Catalog #PD8117**

EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G**

StemPro™ Accutase™ Cell Dissociation Reagent **Thermo Fisher Scientific Catalog #A1110501**

RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

FBS **Invitrogen - Thermo Fisher**

LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation **Thermo Fisher Catalog #L34965**

Click-iT™ Plus EdU Alexa Fluor™ 488 Flow Cytometry Assay Kit **Thermo Fisher Catalog #C10632**

BSA fraction V **Gibco - Thermo Fischer Catalog #15260037**

Alexa Fluor® 647 Mouse Anti-Insulin **BD Biosciences Catalog #565689**

Islet dispersion and fixation 3m

1 **Dispersing islets into single cells** 17m

Wash approximately 100-200 islets two times with ice cold PBS containing **2 millimolar (mM)** EDTA (**8**) in a 1.5 ml microcentrifuge tube using a microcentrifuge at **339 x g, 4°C, 00:03:00** .

Keeping the tubes on ice add **200 µL** - **300 µL** of ice cold Accutase solution.

Transfer the tubes to a water bath at **37 °C** and gently mix the islet suspension using a 1 ml pipette tip during a maximum of **00:10:00** .

Return the tubes to ice and add an equal volume of islet media (eg.10% FBS in RPMI 1640), mix and centrifuge at **339 x g, 4°C, 00:04:00** .

2 Live/Dead staining and fixation

1h 9m

Discard the supernatant and wash the cells once with PBS and suspend in **1 mL** of PBS on **On ice** .

Add **2 µL** (for human islets) or **1 µL** (for rat and mouse islets) of the reconstituted Aqua LIVE/DEAD fluorescent reactive dye, mix and incubate **On ice** for **00:30:00** (**00:20:00** for rat or mouse islets), protected from light.

Centrifuge at **339 x g, 4°C, 00:04:00** and wash once with **1 % (v/v)** BSA in PBS **On ice** .

Suspend the cell pellet and add **100 µL** of Click-iT™ plus EdU kit fixative (Component D), mix and incubate for **00:15:00** at **Room temperature** protected from light.

Add **1 mL** of **1 % (v/v)** BSA in PBS **On ice** , centrifuge as above and wash with **1 mL** Click-iT™ plus EdU kit perm/wash reagent. (To store for up to one week at **4 °C** , perform instead 2 washes with **1 % (v/v)** BSA in PBS **On ice** .)

Staining and flow cytometry 55m

3 Click-iT™ plus EdU reaction

55m

Centrifuge as above and suspend the cell pellet in **50 µL** Click-iT™ plus EdU kit perm/wash reagent and mix. Incubate for **00:10:00** **37 °C** . During the incubation step prepare the Click-iT™ plus EdU kit reaction cocktail.

Start by preparing a 1X Click-iT™ plus EdU kit buffer additive by diluting the 10X stock solution in water.

Then, prepare the Click-iT™ plus EdU kit reaction cocktail at **Room temperature** and use within **00:15:00** .

2 rxn	5 rxn	
875 µL	**2.188 mL**	PBS
20 µL	**50 µL**	Component F
5 µL	**12.5 µL**	488-dye azide
100 µL	**250 µL**	1X Buffer additive

Add **450 µL** of Click-iT™ plus EdU kit reaction cocktail to each tube, mix and incubate for **00:30:00** at **Room temperature** , protected from light.

Centrifuge and wash the cells once with **1 mL** of Click-iT™ plus EdU kit perm/wash reagent.

4 Antibody staining

20m

Centrifuge and remove the supernatant. Dislodge the cell pellet and resuspend in **50 µL** of Click-iT™ plus EdU kit perm/wash reagent containing **2 µL** of Alexa Fluor® 647 Mouse Anti-Insulin and incubate for **00:20:00** at **Room temperature** in the dark.

Wash twice with **1 mL** Click-iT™ plus EdU kit perm/wash reagent and suspend in **200 µL** of **1 % (v/v)** BSA in PBS.

5 Flow cytometry

Dead-cell stain, EdU and insulin labelled cells are detected using 405-, 488-, 640-nm lasers coupled with 525/50-, 530/30-, 670/14-nm BP filters, respectively, or similar. Proliferation is calculated as the

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percentage of double-positive EdU^+ and Ins^+ cells over the total Ins^+ cell population.

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