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Isolation of mouse islet cells, culture with heparan sulfate mimetics and flow cytometry analysis of beta cell viability

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1 Works for me dx.doi.org/10.17504/protocols.io.bmgjk3un

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ABSTRACT

Isolated mouse islets were dispersed into single cells using Accutase (Millipore; 250 µl/500 islets). 4-8 x 10^4 islet cells were transferred to individual wells of a 96 well culture plate (CELLSTAR, Greiner Bio-one) for immediate staining for flow cytometry analysis or for culture prior to staining. Isolated mouse islet cells were cultured in the presence or absence of the HS mimetics heparin (a highly sulfated HS analogue from porcine intestinal mucosa) or PI-88 (Progen Pharmaceuticals Limited,) at 50 mg/ml for 2 days in 5% CO_2, 95% air at 37ºC. In some studies islet cells were acutely treated with 30% H_2O_2 (Chem-Supply) as a source of reactive oxygen species (ROS) for 5 min on day 0 or after culture for 2 days with/without HS mimetics. Damaged and dying islet cells were assessed using Calcein-AM (Calcein; 0.04 µM; Invitrogen)/Propidium iodide (PI; 2.5 µg/ml; BD Biosciences) or by Sytox green (31.25 nmol/L; Invitrogen, Molecular Probes) uptake. BD LSR Fortessa flow cytometer BD FACS DIVA software (version 8) were used to collect events and Flow Jo software (version 10.0.7, TreeStar Inc.) was used to analyse the intensity of fluorescence staining.

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KEYWORDS

mouse beta cells, heparan sulfate, heparan sulfate replacement, beta cell viability

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BEFORE STARTING

Materials:

1. Prepare:

(i) PBS/3mM EDTA:

112 mg EDTA (AJAX #180) in 100 ml PBS, sterile filter using 0.2 µm disposable filter.

(ii) Beta cell culture medium:
RPMI 1640, Sigma #R0883, 200 ml
Heat-inactivated fetal calf serum (HIFCS), 20 ml
L-Glutamine (Gibco # 25030081 200 mM) 2 ml (final 2mM)
Penicillin G, MP Biomedicals #02194537, 0.06 mg/ml
Streptomycin, Sigma #S9137, 0.10 mg/ml
Neomycin, Sigma #N6386, 0.10 mg/ml

2. Other reagents:
Accutase, Millipore #SCR005
Cell culture plates: Cellstar #650180(Greiner Bio-one)
Calcein-AM (Calcein), 0.5mM, Invitrogen #C3100MP
Propidium Iodide (PI), 50 mg/ml, BD Biosciences #556463
SYTOX Green, 5 mM, Invitrogen, Molecular Probes #S7020
Hydrogen peroxide (30% w/w), Chem-Supply Pty Ltd (Australia) #HA154-500M

1 See Guidelines, “Before starting”

2 Isolated mouse islets were transferred to a 15 ml tube and excess medium was removed using a pipette. Resuspend in ~10-15 ml PBS/3mM EDTA. Centrifuge at 249g.

3 Resuspend the islets in PBS/3mM EDTA. Centrifuge at 249g then carefully remove the supernatant.

4 Gently resuspend each pellet inpre-thawed Accutase (250 µl/500 islets) and place tubes in 37°C waterbath for 10 mins (Note: at 4 min and 8 min, gently knock the pellet to resuspend the islets)

5 Dissociate the islets by pipetting up and down 10-15 times using a 1 ml single channel pipette.

6 Add 10 ml culture medium to each tube to terminate the Accutase reaction and centrifuge for 5 min at 249g.

7 Discard the supernatant, resuspend in beta cell culture medium (500 µl/500 islets) and determine cell density (using hemocytometer).

8 Transfer islet cells to culture plate, 4-8 x 10^4 cells /well and adjust the volume in the wells to 200 µl by adding beta cell culture medium. Centrifuge at 249g then remove the supernatant by flicking.

9 Islet cells are cultured with heparin or heparan sulfate mimetics (e.g. PI-88) at a final concentration of 50 µg/ml in 200µl/well. Control cells are cultured in medium.

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10 Cell viability is determined on day 0 and 2 days after culture by staining with Calcein/PI or SYTOX Green followed by flow cytometry analysis:

(i) For Calcein-AM/Propidium Iodide staining, centrifuge culture plate at 110-173g for 3 min and remove culture supernatant. Resuspend cells in 0.04 µM Calcein, 100µl/well. Incubate at 37ºC for 15 min. Add 100µl culture medium, centrifuge at 110-173g. Remove culture supernatant and resuspend in 2.5µg/ml, 100µl/well. Incubate at 37 C for 15 min. Add 100µl PBS, centrifuge at 110-173g. Remove culture supernatant and resuspend in 100µl PBS for flow cytometry analysis. Analyse flow cytometry data using FlowJo software (version 10.0.7, TreeStar Inc.). Viable beta cells are Calcein+ve PI-ve; damaged beta cells are Calcein+ve PI+ve; and dead cells are Calcein-ve PI+ve.

Excitation/emission wavelengths:
Calcein-AM: 494 nm/517 nm
PI: 493 nm/636 nm

(ii) For monitoring hydrogen peroxide-induced cell death, centrifuge culture plate at 110-173g for 3 min and remove culture supernatant. Resuspend cells in 100µl of 30% H_2O_2 or culture medium for 5 min. Add 100µl culture medium and centrifuge at 300g for 3 min. Remove culture supernatant and resuspend cells in 31.25 nM SYTOX Green (1/160,000 dilution of stock), 100µl/well. Incubate at 37C for 15 min. Add 100µl PBS, centrifuge at 110-173g for 3 min. Remove culture supernatant and resuspend cells in 100µl PBS for flow cytometry analysis. Analyse flow cytometry data using FlowJo software (version 10.0.7, TreeStar Inc.). Dead/damaged islet cells are SYTOX Green+ve, compared to unstained controls.

Excitation/emission wavelengths:
Sytox Green: 504 nm/523 nm

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