Human Islet Cell Culture

Bridget Wagner[1]
[1]Broad Institute

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1 Works for me This protocol is published without a DOI.

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bwagner

ABSTRACT

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Cell-culture formulations

1 The HTB-9 bladder carcinoma cell line (ATCC) is cultured in medium containing RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1 x penicillin-streptomycin.

2 Islet culture medium: Islets are cultured in CMRL-1066 supplemented with 10% FBS, 1 x penicillin-streptomycin, and 2mM L-glutamine.

Conditioned medium: Preparation of matrix-coated 384-well plates

3 Culture HTB-9 cells in T-175 flasks until 100% confluent, then incubate for an additional 3 days after changing media.

4 Collect the conditioned HTB-9 supernatant, and filter with 0.22 mm filter and store at -20C until use.

5 The day before you plan to seed islet cells, coat each well of a 384-well plate (PerkinElmer, CellCarrier-384 Ultra) with 10 mL/well HTB-9 supernatant and incubate overnight at 37C, 5% CO2. If you are short of time, 1-2 h is acceptable.

6 Wash each well twice with 50 mL/well PBS.

7 Add 10 mL/well islet culture medium (see above) and return to the incubator in advance of receiving islets.

8 As an alternative, coated plates filled with 50 mL/well PBS (after the same two washes) can be stored at 4C for up to 1 month.

Islet culture in 384-well plates

9 Upon receipt, pellet islets by centrifugation in a 50-mL Falcon tube and wash once with PBS.

10 Dissociate islets in Accutase (5000 IEQ/mL) in a Falcon tube for 20 min at 37C in 5% CO2 incubator, keeping cell clumps down to ~10 cells. After ~10 min, agitate the Falcon tube and return to the incubator.

11 Pipette 5-7 times gently.

12 Add CMRL medium to dilute Accutase (9 mL for every 1 mL Accutase).

13 Resuspend islets in islet culture medium (see above) at a density of 250,000 cells/mL.

14 Dispense 40 mL/well into coated 384-well plates, which will give you 10,000 cells/well. We use the MultiDrop Combi (Thermo Fisher) for semi-automated dispensing.NOTE: this section can be adapted to 96-well format by altering the volumes and cell density. NOTE: this section can be adapted to 96-well format by altering the volumes and cell density.

Cell proliferation

15 After incubating for 24hr at 37C, 5% CO2, aspirate medium, leaving 10 mL/well. We use the BioTek ELx-405 automated plate washer for this step. Unless otherwise indicated, we aspirate medium so that 10 mL remains, and then add 40 mL/well to bring the total to 50 mL.

16 Add 40 mL/well medium containing 12.5 mM EdU (final concentration = 10 mM). Total volume is 50 mL/well.

17 Treat and incubate according to your experiment. We transfer compounds from compound plate (Abgene 384 PP, V-bottom) to cell plates using the Cybio Well Vario (Analytik Jena). We have found that the final DMSO concentration should remain ≤ 0.3%.

Change the culture medium containing 10 mM EdU (and add compounds as appropriate) every two days.

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19 Aspirate the medium, then add 40 mL/well 4% PFA/PBS (final concentration will be 3.2%) at room temperature for 20 min.

20 Wash cells twice with PBS.

21 Permeabilize islet cells with 0.2% TritonX-100/PBS (final concentration) at room temperature for 20min.

22 Wash twice in PBS.

23 Stain cells using the Click-iT EdU HCS assays (catalog number C10351, Invitrogen) according to the manufacturer's protocol.

24 Block with 2% BSA/PBS at room temperature for 2 hr while gently shaking. No need to wash after this step.

25 Incubate primary antibody (C-peptide) at 1:100 dilution in 2% BSA/PBS overnight at 4C with gentle shaking.

26 Wash cells three times with PBS, then once with 2% BSA/PBS.

27 Incubate with Alexa Fluor 594-conjugated secondary antibodies (1:1000 dilution) and HCS NuclearMask Blue stain for 1 hr at room temperature.

28 Wash cells five times in 80 mL/well PBS. On final wash, leave wells filled with 50 mL/well PBS.

29 Seal plates with foil seal and store at 4C before imaging.