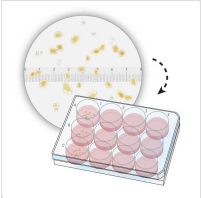

Jan 10, 2022

Static Incubation of Pancreatic Islets

Islet and Pancreas Analysis Core[1]

[1]Vanderbilt Diabetes Research Center

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Vanderbilt Diabetes Research Center

Islet and Pancreas Analysis Core
Vanderbilt Diabetes Research Center

This SOP defines the methods used by the Vanderbilt Diabetes Center Islet and Pancreas Analysis (IPA) Core for static incubation of pancreatic islets isolated from mouse or human tissue. See also our islet isolation protocol.

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In steps of

Pancreatic Islet RNA Extraction

Reagents and supplies:

- RPMI 1640 (Gibco 11879020)
- Penicillin/streptomycin (Mediatech MT30002CI)
- Glucose (Fisher Scientific D16)
- Fetal bovine serum (Millipore Sigma F0926)
- DMEM powder (Corning MT90113PB)
- Sodium bicarbonate (Sigma S6014)
- L-glutamine (Sigma G8540)
- HEPES (Sigma H7523)
- Sodium pyruvate (Sigma P2256)
- Bovine serum albumin (Sigma A7888)
- Ascorbate (Sigma A5960)
- Deionized water
- 95% ethanol
- Hydrochloric acid (Fisher A508)
- 12-well untreated cell culture plates (Corning 351143)
- 6 cm untreated cell culture dishes (Sarstedt 83.3901.500)
- 0.22 micron vacuum filter (Millipore SCGPU05RE)
- 2 mL screw-top tubes (VWR 89004-290)

General lab supplies:

- 5 mL serological pipet (Fisher Scientific13-678-11D)
- 1 mL micropipette tips (Fisher P-2079E)
- P-200 micropipette tips (Fisher Scientific 212361)
- 500 mL plastic storage bottles (Fisher 09-761-10)
- 15 mL conical tubes (Fisher 12-565-268)
- 2 mL microcentrifuge tubes (Fisher 05-408-129)
- 1 L Erlenmeyer flask
- Magnetic stir bar

Equipment:

- Pipet filler (Fisher Scientific S-1)
- Analytical balance
- BSL-2 biological safety cabinet
- Magnetic stir plate
- Inverted microscope with reticle
- P-300 electronic pipette (Eppendorf Xplorer or similar)
- Stereomicroscope (Olympus SZX12 or similar)
- P-1000 micropipette (Eppendorf Research Plus or similar)
- Centrifuge (Eppendorf 5702R or similar)
- Cell culture incubator set to 37°C, 5% CO_2

Reagent preparation

1 **RPMI with 5.6 mM glucose** (cell culture media):

- Remove 5 mL from a 500 mL bottle of RPMI, and add 5 mL penicillin/streptomycin. Close

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bottle and invert to mix.

- Add 0.505 g glucose to RPMI, invert to mix, and incubate at room temperature for **01:00:00** to allow glucose to go into solution.
- In a BSL-2 culture hood, vacuum filter 450 mL RPMI.
- Add 50 mL FBS.

Once made, media can be stored at **4 °C** for up to 1 month.

2 **DMEM** (static incubation media):

Add the following ingredients into a 1 L Erlenmeyer flask.

- 8.28 g DMEM
- 3.2 g sodium bicarbonate
- 0.58 g L-gutamine
- 1.11 g HEPES
- 0.11 g sodium pyruvate
- 1 g bovine serum albumin
- 70 mg ascorbate
- 1 L deionized water

Place a stir bar into the flask and situate on top of a magnetic stir plate. Stir at medium-high speed for **00:15:00** to allow all ingredients to go into solution.

2.1

Aliquot 500 mL of DMEM media into each of 2 plastic storage bottles to make 5.6 mM glucose and 16.7 mM glucose solutions.

2.2 **DMEM with 5.6 mM glucose:**

- Add 0.505 g glucose to 500 mL prepared DMEM media.
- Incubate for **01:00:00** to allow glucose to go into solution.
- Vacuum-filter media.

2.3 **DMEM with 16.7 mM glucose:**

- Add 1.504 g glucose to 500 mL prepared DMEM media.
- Incubate for **01:00:00** to allow glucose to go into solution.
- Vacuum-filter media.

3 **Acid ethanol (for hormone extraction):**

- Pipet 5.5 mL 95% ethanol into a 15 mL conical tube.
- Add 50µL hydrochloric acid.

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- Invert to mix.

Static incubation

4 Pipet 5 mL **RPMI with 5.6 mM glucose** into each of two 6-cm cell culture dishes.

5 Using an electronic pipette and an inverted microscope, pass islets into the first plate, and then into the second.

6 Place islets into a **37 °C** cell culture incubator with 5% CO_2 and leave overnight for a minimum of **18:00:00** .

7 Pipet 5 mL of **DMEM with 5.6 mM glucose** into a 6-cm cell culture dish.

8 Using an electronic pipette and an inverted microscope, transfer islets from overnight incubation plate (step 6) into the 6-cm plate with DMEM media. Islets will be picked from this plate and placed into the 12-well static incubation plate described in steps 9-10.

9 Label a 12-well plate for static incubation according to your experimental design. In the example below, two conditions are shown, low glucose (5.6 mM) and high glucose (16.7 mM). These conditions can be repeated in subsequent rows and/or plates for biological replicates as desired.

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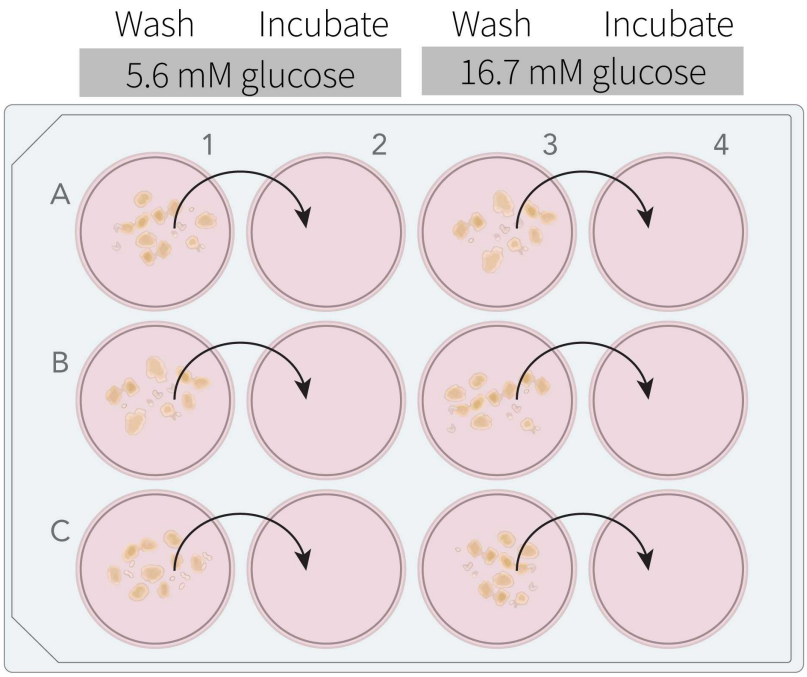
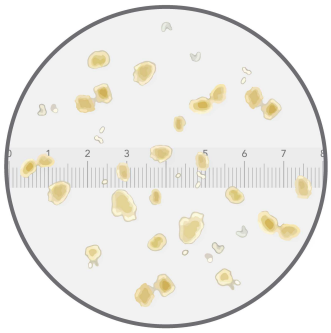


Figure 1: Example schematic of a 12-well incubation plate.

9.1 Pipet 2 mL of the appropriate media into each well. Note that **each condition requires two wells**, a "wash" well and an "incubation" well.

10 Under microscope guidance, place around 20 islet equivalents (IEQs) into each wash well. This is typically a combination of 12 small, 5 medium, and 2 large islets. Use the chart below to measure islet sizes.



Using reticle calibrated to 25-µm increments:

Islet size category	Number of tick marks	Islet diameter (µm)
Small	>4 and ≤6	>100 and ≤150
Medium	>6 and ≤8	>150 and ≤200
Large	>8 and ≤10	>200 and ≤250
Extra large	>10 and ≤12	>250 and ≤300

Figure 2: A reticle is used to IEQ islets and aliquot them into 12-well incubation plates.

The total volume of isolated islets is generally expressed as an **islet equivalent** or 'IEQ,'

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defined as an islet of 150-µm diameter. This helps to normalize downstream measurements, accounting for the fact that smaller islets contain fewer cells. *As such, if islets are on the smaller side, 20 IEQ will consist of >20 individual islets; if islets are larger, 20 IEQ may consist of <20 individual islets.*

11 Once islet aliquots have been distributed to wash wells, place the 12-well plate onto the stereomicroscope stage, swirl the plate to move islets to center of each well, and transfer the islets into the subsequent incubation wells. Use no more than 20 µL media for the transfer to avoid dilution of culture media.

12 Cover the 12-well plate and place in **37 °C** incubator with 5% CO_2 for **01:10:00** .

The first 10 minutes of the incubation are designated as "warm-up" time, so this protocol is considered a 1-hour incubation.

Media collection and hormone extraction

13 Remove plate from incubator and place on ice to cool down for **00:10:00** . During this time, label two 2-mL microcentrifuge tubes for each incubation well (islet aliquot): the first for islet extract and the second for media.

14 Return plate to the stereomicroscope stage, swirl the plate to move islets to center of each well, and pipet the islets in each incubation well into the first of each pair of labeled microcentrifuge tubes.

15 Centrifuge the islet tubes for **00:03:00** at **200 rcf** .

16 Remove supernatant from islet pellets, and add 200 µL **acid ethanol** to each tube.

17 Transfer islet extract tubes to **4 °C** to begin incubation for **24:00:00** . Meanwhile, proceed to step 18.

18 After islets are removed, transfer 1.5 mL media from each incubation well into the second of

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each pair of labeled microcentrifuge tubes. Store at **-20 °C** .

19 Following the **24:00:00** incubation in step 17, spin down islet extract tubes for **00:05:00** at **3000 rcf** .

20 Transfer three 50-µL supernatant aliquots from each islet extract tube into prelabeled 2-mL screwcap tubes and store at **-80 °C** .

21 Perform hormone assay of your choice.

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