Full Media Change for Neonatal Porcine Islets

Performed on the first and third day after isolation

Equipment:

- 50 ml and 250 ml conical tubes
- 10 ml, 25 ml and 50 ml serological pipettes
- 150 mm Ø plates

Buffers:

- Hanks Balanced Salt Solution (HBSS), warmed to 37 °C supplemented with 0.25 % bovine serum albumin, 10 mM Hepes, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.
- Ham's F-10 (warmed to 37 °C) supplemented with 10 mM glucose, 50 µM IBMX, 0.5 % bovine serum albumin, 2 mM L-glutamine, 10 mM nicotinamide, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.

Procedure:

1. Label 4 plates with appropriate numbers for the pancreases and fill plates with Ham's F-10 cell-culture medium (first plate with 20 ml, next three with 30 ml each). Place the plates in an incubator at 37 °C to equilibrate in stacks of 4 with the 20 ml plate on the bottom.

2. Fill 50 ml conical tubes with 30 ml of Ham's per pancreas and warm to 37 °C (i.e. 4 pancreases will require 120 ml of Ham's).

3. Fill 2-4 tubes with warmed HBSS for convenience.

4. Combine all four plates of one pancreas in a 250 ml conical tube:

   a) Take up 10 ml of the cell suspension, rinse the tilted plate, then take up all 35 ml and transfer to the 250 ml tube.

   b) Rinse the plate with 10 ml of HBSS – if cells still appear to be in the plate, rinse again (usually two rinses are required).

5. When the islets of two pancreases have been transferred to their respective 250 ml tubes, fill the tubes to the top with HBSS and cap.

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6. Collect the islets of a third pancreas and transfer to a 250 ml tube as above. Top with HBSS and cap.

7. Take the first two 250 ml tubes of islets and centrifuge at 160 g and room temperature for 1 min. During this spin time, collect the islets of the fourth pancreas as above. This collection should take about the same time as the spin, so the first two tubes can be traded for the second two so there is no down time.

8. For the first two pancreases, remove most of the supernatant, leaving approximately 20 ml behind with the cells.

9. Resuspend the cells and take up the 20 ml cell suspension. Transfer it to a 50 ml tube, rinse the 250 ml tube at least two times with HBSS and add to the 50 ml tube.

10. Fill the 50 ml tubes with HBSS and centrifuge at 160 g for 1 min. Steps 8 and 9 should take about as long as the spin, so again the 50 ml tubes for pancreases 1 and 2 are traded for the 250 ml tubes of 3 and 4.

11. Repeat steps 8-10 for the pancreases 3 and 4, trading the 50 ml tubes for the 50 ml tubes of 1 and 2.

12. Remove the supernatant of pancreases 1 and 2 and resuspend the islets in a total volume of 20 ml of the warmed Ham's (from step 2) by adding 8 ml of Ham's with a 10 ml serological pipette to each tube, then taking up the total volume and recording the cell volume. Then add 12 ml more of Ham's less the cell volume (this ensures that the cell + Ham's suspension are less than the volume of Ham's added after so the pipette is being rinsed).

13. Step 12 should take less time than the spin, so get the plates at this time.

14. Repeat step 12 for pancreases 3 and 4.

15. Put 5 ml of the cell suspension in each of the equilibrated plates. Rinse the tube with 10 ml of-Ham's and add to the plate that had 20 ml of Ham's initially.

Half Media Change for Neonatal Porcine Islets

Performed on the every other day after the first 3 days post-isolation

Equipment:

- 10 ml serological pipette
- 15 ml conical tubes

Buffers:

- Ham's F-10 (warmed to 37 °C) supplemented with 10 mM glucose, 50 µM IBMX, 0.5 % bovine serum albumin, 2 mM L-glutamine, 10 mM nicotinamide, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.

Procedure:

1. Label 15 ml conical tubes with appropriate numbers for the 4 plates of one pancreas.

2. Warm 15 ml of Ham's per plate to 37 °C.

3. Use a 10 ml serological pipette that has been previously coated with a media containing BSA (i.e. take up ~13 mL of HBSS or Ham's – this is to ensure the islets don't stick to the inside of the pipette).

   Take up the full volume of the pipette and transfer to the appropriate 15 ml conical tube. (Gently swirling the plate in a circular motion until the islets gather in the centre of the plate minimizes the number of islets that will be transferred to the tube.)

4. When all 4 15 ml tubes have been filled, centrifuge at 160 g and room temperature for 1 min

5. Remove most of the supernatant, leaving approximately 0.5 ml behind with the cells.

6. Resuspend the pellet in 10 ml of the warmed Ham's.

7. Transfer the 10 ml back to the numbered plate, rinse the tube with 5 ml of Ham's and transfer the rinse to the plate as well.

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