Protocol for Mouse Islet Isolation

Isolation Buffer:
HBSS containing 10mM of HEPES, 1mM of $MgCl_2$, 5mM of Glucose, pH 7.4

Washing Buffer: Isolation buffer containing 1mM $CaCl_2$

Collagenase P: Boehringer Mannheim-Roche (CAT# 1213873)

Equipment needed:
Hemostat clamp: n=2
Forceps: n=2
Scissors: large x1 for skin incision, small x1 for taking out pancreas from adjacent tissue
5 ml syringe: need the same number as the number of mice.
30 G needle: need the same number as the number of mice.
Sterile siliconized glass vials w/Teflon cap (Fisher, cat#:213-018-54) need the same number as the number of mice.
Sterile large sink strainer: n=1
Sterile 1000 ml Beaker: n=1
Gauze 4x4 pad: 2/mouse
Gloves
Spoide (sterile plastic eye dropper)

Collagenase Preparation:

1. Weigh out the collagenase into a 50 ml conical and add **isolation buffer** to make the final concentration as indicated in the table below. Dissolve the collagenase by vortexing. (12 mice + 2 extra mice = 14 mice x 5 ml/mouse = 70 ml total collagenase solution) (70 ml x 0.5 mg/ml or 0.3 mg/ml = 35 mg or 21 mg of collagenase P total)

Strain	Age	Collagenase P (mg/ml)	Time (min)
C3H, Balb/C, B6	> 12 weeks	0.8	17
C3H, Balb/C, B6	<= 12 weeks	0.5	13
NOD, NOR, NON	> 12 weeks	0.8	17
NOD, NOR, NON	<= 12 weeks	0.5	17

2. Dispense 2 ml of collagenase solution into each glass vial and place on ice. Load each 5 ml syringe with 3 ml of collagenase add 30g needle and place in ice (prepare in hood).

Distension:

3. Just before dissection, sacrifice animal by cervical dislocation. Spray the whole body with 70% of ETOH making it completely wet. Make a V-insision starting at the genital area. Rotate the mouse so that the tail is facing away from you.
4. Remove the bowel to the left side of the open mouse. This will expose the pancreas and common bile duct.
5. Place a hemostat clamp on either side of, the small-intestinal, where the bile duct drains, leaving a small pocket for collagenase solution to enter the intestine.
6. Inflate the pancreas through the bile duct with a 30g needle and 5 ml syringe containing 3 ml of cold collagenase solution, starting at the gall bladder.

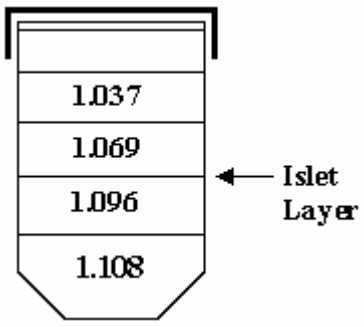
7. Remove the pancreas from the body and place it in a siliconized vial containing 2 ml of collagenase solution. This step should be done **QUICKLY** and cleanly as possible, minimizing collection of fat and connective tissue.
8. Place the vial on ice and repeat step 3, with the next mouse.

Digestion:

9. Seal each vial and place it into a 37^{O}C water bath. Incubate 13-17 minutes (time varies with strain and age of the animal).
10. After time has elapsed, shake the vial vigorously. The pancreas should fall apart.
11. Pour each digest through a large sterile sink strainer into a sterile 1000 ml beaker and pipette off the screen with washing buffer forcefully. Dispense the digests into 50 ml conicals (3 pancreata/50 ml conical: if n=12, use enough washing buffer to make the final volume 200 ml and dispense it to 4x50 ml conicals). The dilution should be done ***rapidly and on ice*** to avoid unwanted islet digestion.
12. Spin down: Start the centrifuge. When it reaches 1300 rpm, turn it off.
13. Aspirate the supernatant, leaving ~5 mL. Be very cautious not to aspirate the pellet.
14. Resuspend pellet by tapping vigorously with your hand, then add 50 ml of wash buffer.
15. Spin down: Start the centrifuge. When it reaches 1300 rpm, turn it off.
16. Resuspend the pellet and wash with 5 ml of washing buffer.
17. Transfer the 5 ml to 15 ml conical and spin down, as above.
18. Aspirate the supernatant as completely as possible. (Remaining buffer might cause the change of the density of the Ficoll.)

Ficoll: ***Perform quickly. Long-term Ficoll exposure is TOXIC to islets.***

19. Make sure pellet is completely broken up prior to adding ficoll. (Unbroken tissue is difficult to resuspend in ficoll)
20. Resuspend the pellet in 7 ml of ficoll, density 1.108 by vortexing vigorously.
21. Layer on top of each density of ficoll 2ml of each of the remaining densities in this order: 1.096, 1.069, then 1.037.
22. Spin for 15 min at 1800 rpm at 4 degrees **with break OFF!**
23. Pick islets from the second layer using spoid (sterile plastic eye dropper). Transfer all collections to a 50 ml conical containing ~25 mL of **cold** buffer.
24. Wash as above 3 times with washing buffer (repeating step 12-15).
25. Resuspend Islets in 20 ml RPMI-1640 (containing 10% FCS and penicillin and streptomycin, HEPES, MEM-NEAA) and mix gently. Remove 100 ul of sample for counting.
26. Transfer 100 ul to 35 mm Grid-plate containing 1 ml of media and 1 ml dithizone.
27. Incubate remaining islets in RPMI 1640 in a 37^{O}C, 7% CO_2 Incubator. In 160 mm plates with a total of 30 ml of media/plate.



Dithizone:

5 mg Dithizone (Diphenylthiocarbazone)
5 ml DMSO
45 ml Normal Saline or HBSS

- Dissolve Dithizone in 5 ml of DMSO in a 50 ml conical.
- Add 45 ml of normal saline and mix gently.
- Filter with a 60 cc syringe and a 0.22 μm syringe filter into a new 50 ml conical.
- Store at 4 ^{O}C, until use.
- Add 1 ml to each 35 mm counting Grid-plate, when needed.