**Isolation of Mouse Islets**

*Procedure for collecting islet cells from mice. These cells can be kept as whole islets or dissociated into beta cells for experiments.*

**RPMI full medium for islet culturing:**

* + RPMI 1640 with L-Glutamine 500ml (Gibco 11875-093)
  + FBS 50ml (Gibco 26140-079)
  + Pen/strep 5ml (Gibco 15140-122)
  + Sodium Pyruvate 5ml Gibco 11360-070)

**Mouse Dissection Buffer (1.5L is enough for about 6 mice):**

|  |  |  |  |
| --- | --- | --- | --- |
| **Liters** | **1** | **4** | **5** |
| 10x HBSS | 100 | 400ml | 500ml |
| HEPES | 2.38g | 9.52g | 11.9g |
| 1M MgCl2 | 1 ml | 4 ml | 5 ml |

\* Autoclave after prep

\*\* Check pH after mixing, should be around 7.4, adjust if necessary using NaOH or HCl

\*\*\* Can substitute with RPMI with HEPES

* On the day of isolation, to 1L mouse buffer, add:
* 0.2g Bovine Serum Albumin Fraction V Protease-Free (Roche; Ref 03117332001)BSA and
* 5ml of 250mM CaCl2
* Prepare 0.5mg/ml Collagenase P solution (Roche Ref. 11249002001) in Dissection Mouse Buffer with BSA and CaCl2 (5ml and 2.5mg Coll-se P per mouse)

**Procedure for collecting pancreatic islets from mice.**

1. Prep Mouse Buffer and collagenase (see solution prep info above)
2. Collect bucket of ice and place the mouse buffer in it.
3. Fill syringes with 5ml of collagenase solution each and attach 30G (1/2 in) needles, place on ice
4. Place 1.5ml of mouse buffer into 50ml conical vials (1 per mouse), place on ice
5. Inject and remove pancreas per protocol and place into the labeled conical 50ml vial
   1. Save these on ice until 2 mice are done.
6. Put labeled vials into 370C water bath for 10 minutes
7. Shake with arm swings (~30 times)
8. Fill vial to the top with mouse buffer and remove non-pancreatic tissue that floats to the top, including spleen and fat
9. Centrifuge for about 15 seconds
10. Suction off the top fluid, leave the pellet untouched
11. Add about 30mls of buffer to the pellet
12. Vortex on bench for 10 seconds
13. Fill with buffer to 50 ml
14. Centrifuge for ~15 seconds
15. Suction to pellet
16. Have a 50ml conical vial with mouse buffer in the hood
17. Have two 15ml conical vials labeled for each mouse sample
    1. One empty
    2. One with 4mls of Histopaque in it
18. Add **Histopaque 1119 (Sigma H1119)** about 8ml to 50ml conical vials after having suctioned them down to the pellet
19. Vortex for 10 seconds
20. Pour into the empty 15ml conical vials
21. Add about ~1mls of mouse buffer, slowly, to the top of vial and you might see the islets float to the top
22. Centrifuge x 20-25 seconds
23. Collect islets from the mid-layer of the vial and place into the conical vial with the 4 mls of Histopaque in it (pipet up and down to break up)
24. Add 1-2mls of buffer to the top of the tubes, slowly, and centrifuge x20 seconds
25. Collect islets with pipet from the mid-layer of the vial and place into 50ml conical vials with mouse buffer
26. Centrifuge 15 seconds
27. Suction off the buffer, be careful, this pellet is very delicate and small
28. Label plates (2/sample)
29. To one of the two plates, add 10ml of RPMI medium
30. To conical vial with pellet, add 10ml of RPMI medium, mix, and pour onto the empty plate
31. Place dish with clean medium off to the side of the microscope
32. Rotate dish containing islets to gather them on one side
33. Pick islets using pipet under inverted microscope and place them on the clean plate with full RPMI medium.
34. Once complete, throw away the plate on the microscope and bring your picked islets to the incubator.