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Protocol for Recovering the Islets after Receiving in Transport Solution

1. **PURPOSE:**
   This protocol describes how to retrieve islets after receiving in transport media.

2. **MATERIALS REQUIRED:**
   1. PIM(S)™ media (Prodo labs – Cat no. - PIM-S001GMP).
   2. PIM(ABS)™ (Prodo labs – Cat no. - PIM-ABS001GMP).
   3. PIM(G)™ (Prodo labs – Cat no. - PIM-G001GMP).
   4. Shipping package with bottle containing islets.
   5. 250mL centrifuge tubes (Corning® 250mL PP Centrifuge Tubes with Plug Seal Cap, Sterile (Product #430776) or equivalent) OR 50mL centrifuge tubes (VWR® High-Performance Centrifuge Tubes with Flat or Plug Caps, Polypropylene, Sterile (Cat no. -89039-658) or equivalent).
   6. 60mm petri dish (VWR® Petri Dishes, Contact Plate, Sterile (Cat n 25384-093) or equivalent).
   7. T-150 non-tissue culture treated flasks (Corning * Non-Treated Culture Flasks, Polystyrene, Sterile, (Cat no. 431465 or equivalent)).

3. **PROCEDURE:**
   To avoid or minimize the chance of contamination, the steps below are performed in a laminar flow hood with good sterile technique.

   1. Supplement the 500mL bottle of PIM(S) media with 25mL of PIM(ABS) and 5mL of PIM(G).
   2. Cool the PIM(S) (Prodo labs – PIM-S001GMP) complete media in a 6-10^{o}C refrigerator prior to receiving the islets.
   3. Retrieve the bottle containing islets from the shipping package and keep at 6-10^{o}C until ready to wash.
   4. Pre-wet the appropriate size centrifuge tube(s) that are going to be used for pooling the islets with complete PIM(S) media.
   5. Transfer the islets form the transport bottle using an appropriate size pipette pre-wetted by complete PIM(S) media in to the centrifuge tube(s)
   6. Wash the transport bottle twice with appropriate amount of complete PIM(S) media making sure no islets are left behind in the transport bottle.
   7. If there are any clumps visible (Sometimes during transport islets clump together) using a 10ml pipette pre-wetted with PIM(S) medium, break up the clumps- by very gently aspirating up and delivering the media containing islets close to the conical wall, not touching the wall. Make sure the islets are well dispersed, and there are no visible clumps present.
   8. Bring the volume up to the neck in the centrifuge tube(s) containing the islets, with complete PIM(S) media.
   9. Centrifuge the tube(s) at 180g for 2 mins.
   10. Aspirate the supernatant leaving approximately about 2-3ml with the pellet.

11. Disrupt the pellet by gently tapping on the centrifuge tube. Using a pre wetted 10ml pipette, deliver approximately 5ml of PIMS media and break up any visible clumps by very gently aspirating up and delivering the media containing islets close to the wall and not touching the wall. Make sure the islets are well dispersed, and there are no visible clumps present.

12. Add the desired amount of complete PIM(S) media
13. Take samples for counting and viability and place them in a 60mm petri dish for assessment.
14. Culture at 10K IEQ/T150 flask with 40ml of complete PIM(S) media.