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:

- TM0199-A
- Shipping package with bottle containing islets
- 250 mL centrifuge tubes - Corning® 250 mL PP Centrifuge Tubes with Plug Seal Cap, Sterile (Product #430776) OR 50 mL centrifuge tubes - VWR® High-Performance Centrifuge Tubes with Flat or Plug Caps, Polypropylene, Sterile (Cat no. -89039-658)
- 60 mm Petri dish - VWR® Petri Dishes, Contact Plate, Sterile (Cat no. 25384-093)
- T-150 non-treated tissue culture flasks - Corning * Non-Treated Cell Culture Flasks, Polystyrene, Sterile, (Cat no. 431465).

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) Make TM0199-A Complete Medium according to instructions.
2) Cool the TM0199-A Complete Medium in a 6-10 °C refrigerator prior to receiving the islets.
3) Retrieve the bottle containing islets from the shipping package and keep at 6-10 °C until ready to wash.
4) With complete TM0199-A medium pre-wet the appropriate size centrifuge tube(s) that are going to be used for pooling the islets.
5) Using an appropriate size pipette pre-wetted with complete TM0199-A medium, transfer the islets from the transport bottle into the centrifuge tube(s).
6) Wash the transport bottle twice with appropriate amount of complete TM0199-A medium, making sure no islets are left behind in the transport bottle. Pool islets in centrifuge tube(s).
7) Sometimes islets clump together during transport. If there are any clumps visible, using a 10 ml pipette pre-wetted with TM0199-A medium, break up the clumps by very gently aspirating up and delivering the medium 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, using complete TM0199-A medium.
9) Centrifuge the tube(s) at 180 x *g* for 2 min.
10) Aspirate the supernatant, leaving approximately 2-3 ml with the pellet.

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11) Disrupt the pellet by gently tapping on the centrifuge tube. Using a pre-wetted 10 ml pipette, deliver approximately 5 ml of TM0199-A medium and break up any visible clumps by very gently aspirating up and delivering the medium 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 TM0199-A medium.
13) Take samples for counting total and viable cells and place them in a 60 mm Petri dish for assessment.
14) Culture at 10K IEQ per T150 flask with 40 ml of complete TM0199-A medium.

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