MOUSE PANCREATIC ISLETS ISOLATION KIT

Enzymes

COL G (60 U) recombinant collagenase class I + COL H (255 U) recombinant collagenase class II + Thermolysin (150 µg)

Stock solutions preparation

1. Dissolve COL G in 900 µl sterile H_2O and make 3 aliquots of 300 µl (each aliquot is the Solution A) and store at -20°C

2. Dissolve COL H in 300 µl sterile H_2O and make 3 aliquots of 100 µl (each aliquot is the Solution B) and store at -20°C

2. Dissolve the Thermolysin in 150 µl sterile H_2O and make 3 aliquots of 50 µl (each aliquot is the Solution C) and store at -20°C.

Isolation of Pancreatic Islets from 1 mouse pancreas

DIGESTION SOLUTION for 1 mouse (weight between 20-25 gr): **Solubilize one aliquot of Solution A, one aliquot of Solution B in 5 ml RPMI 1640 and put it on ice. <u>Immediately before use add one aliquot Solution C</u>.**

<u>Prepare all equipment and media before euthanizing the islet donor mouse.</u>

1. Autoclave or flame sterilize instruments before use. All reagents and instruments (touching the samples) should be sterile. The isolation and hand picking of islets can be performed outside of a laminar flow hood without greatly increasing the incidence of contamination. However, sterilization of tools that come into contact with pancreas or islets is critical for avoiding contamination. The following tools are needed:

• 1 pair of dissecting scissors for abdominal incision.

• 2 pairs of forceps.

• 1 hemostatic forceps to clamp off the bile duct.

• 0.419 mm diameter wire mesh.

• 30 gauge needles

• 70% ethanol.

• dissecting microscope with an adequate light source on a lab bench or in a hood.

• RPMI 1640 with 10% serum (cold 4/8°C)

• RPMI 1640 without serum (cold 4/8°C)

• 50 ml conical tubes

• 5 ml syringes

• Histopaque1077 (cold 4/8°C)

• Histopaque 1119 (cold 4/8°C)

Surgical Procedure

Prepare mouse for cannulation. First euthanize the mouse by cervical dislocation or CO2 asphyxiation. While mouse is expiring, add the Solution C to the digestion solution and then fill the 5 ml syringe with 2,5 ml digestion solution and leave on ice. Place mouse in supine position on a paper towel at the dissecting scope and spray the abdomen with 70% ethanol before opening its abdomen with a door shape incision across the pubic region towards both hips, then up along the mouse's right flank to the right front leg and across the thoracic cavity to the left leg. Fold skin over to the right to reveal the abdominal cavity. Now cut away the ribcage and sternum to facilitate greater access to the bile duct.

1. Position the mouse with the head pointing towards you. Reposition the liver by pressing it up against the diaphragm, so that the entire length of the common bile duct is exposed. Locate the duodenal entry of the common bile duct. This can be done without looking through dissecting scope objective by turning back the intestines and distending the duodenum.

2. Clamp the duodenal opening with haemostat. Clamp position is critical for blocking the flow of enzyme solution into the intestines. If clamp is too high or too low, solution will not perfuse the pancreas. Position haemostat, so that when compressed, it runs exactly along the pancreatic/intestine boarder. Clamp, also, the ducts close to the liver.

3. Once the length of the bile duct is exposed, use the 30-gauge needle attached to the digestion solution filled syringe for cannulation.

4. The haemostat clamped on the duodenum is pulled away from the head of the mouse towards the tail so that the common bile duct becomes taut. There is a confluence in the bile duct close to the liver where bile draining from the gall bladder and enzymes from the liver come together before entering the intestines. The inside of the V formed by this confluence is exposed by pulling the bile duct tight and is an ideal place to initiate cannulation of the duct. If positioned correctly, the needle will slide right through the V, and directly cannulate the lower portion of the duct. Insert the needle several millimeters into the duct before dispensing the enzymes solution. Optimal islet yield occurs when your needle depth is far enough that no enzymes solution escapes to the liver/gall bladder but not so far that you've passed the splenic duct.

5. As the 2,5 ml of enzymes solution fills the pancreas, the area near the duodenum begins to expand first, followed by the region on top of the stomach, and finally the splenic tail. If one area begins to expand and you don't see the white tissue evenly expanding and spreading out, it is likely that the common bile duct has not been cannulated, yet the needle is inside the pancreatic capsule. Filling the capsule with digestion solution will not result in a high islet yield as the surface area exposed to the enzyme will be low. If this occurs, stop perfusion and reposition the needle. It may be necessary to cannulate the narrow branch of the pancreatic duct and inject the final 1 ml of digestion solution here for full perfusion.

6. Once the pancreas has been perfused, it can be removed from the mouse by pulling it free from the points of contact with the intestines, stomach, and spleen. Start by removing the haemostat from the duodenum. Then, use the forceps to lift the duodenum and separate the pancreas from the intestines with a second pair of forceps. This is done by holding the second pair of forceps steady while pulling the intestines out of the abdomen. Next, pull the pancreas free from the top of the stomach and the spleen. Finally, lift the pancreas out of the abdomen and cut it free from the remaining fascia connections.

7. Place the pancreas in a molecular biology 10-cm petri dish. It is not recommended to leave the perfused pancreas on ice for more than 1 hour, as the enzymes solution will begin to degrade the tissue.

Purifying Islets from the Perfused Pancreas

Before the islets can be purified from the pancreas, the tissue must be digested at 37°C. Put the pancreas into the 50ml tubes, containing the other 2,5 ml of digestion solution, bearing the perfused islets in an open bottom rack that fits into the 37°C water bath. Ensure that all caps are well secured and submerge tubes in the 37°C water bath for 8/10 minutes with shaking at 100 rpm (after 5 minutes take small sample to stain with dithizone to monitor the digestion, before stopping digestion at the best time). At the end of the incubation, move the tubes to ice and add RPMI1640 (cold 4/8°C) with 10% serum to 20 ml per tube. Serum containing media, partially, will stop the enzyme digestion.

1. Disassociate the tissue by shaking the tubes vigorously 40 times in 10 seconds. This step is critical for optimal recovery of islets. Even with optimal digestion solution perfusion and tightly calibrated digestion time, islet yield will be low if the tissue is not broken up. The aggressive handling of the samples in this step does not appear to harm the mouse islets and is necessary to free them from exocrine cell clusters.

2. To separate the islets from the digested pancreas, complete the following steps:

    1. Centrifuge tubes for 2 minutes at 800 RPM and 4°C.

    2. Pour off the supernatant carefully without disturbing the pellet and resuspend the pellets in 20 ml media (cold 4/8°C) with 10% FBS by up-down through 10 ml sterile pipet, Vigourosly several times (3-5 times). If the digestion is good, it should be easy to resuspend the pellet at this stage.

    3. Pour the resuspended slurry through the 0.419 mm wire mesh into a fresh petri dish. This separates out the non-digested tissue, fat and lymph. Rinse the initial tube with an additional 10 ml of media and pour through the wire mesh over the non-digested tissue. Transfer the filtrate to a fresh 50 ml tube and rinse the petri dish with 5 ml media.

    4. At this stage a 1ml sample of the islet prep can be taken and examined by microscopy. Add dithizone (dissolved 20 mg of dithizone in 2 ml DMSO and after adding 20 ml of RPMI filtered through 0.2 μm) 1:1 to the islet prep and incubate for 10 min then examine by microscopy. Islets (stained red) should be visible and relatively free from the exocrine tissue.

    5. Centrifuge the tubes for 2 minutes at 800RPM and 4°C.

    6. Pour off the supernatant and carefully invert the tubes on a paper towel or aspirate using a vacuum. Watch to make sure that the islets don't slide off. Wipe the inside of the tubes with a paper towel to remove residual media, being careful not to disturb the cell pellet. Return tubes to an upright position.

    7. Dissolve digested tissue in 15 mL ice-cold heavy Histopaque (1.119 g/mL). Transfer to a new 50 mL tube. Ensure that the suspension is homogeneous before adding carefully an additional 20 mL-layer of ice-cold light Histopaque (1.077 g/mL) using a pipetting aid adjusted to minimum power.

    8. Overlay histopaque carefully with 15 mL of ice-cold serum free RPMI 1640, being careful to maintain a sharp interface between the histopaque and media.

9. Spin for 5 min at 800 x g at 4°C. Use slow acceleration (minimal) and prolonged breaking time (maximum). The islets will migrate to the interface between the serum free media and Histopaque (1077) while the exocrine cells will form a pellet at the bottom of the tube.

10. Harvest purified islets from the light histopaque/Medium interface using a pipetting aid and collect in a 50 mL-centrifuge tube prefilled with 15 mL cold RPMI.

11. Fill-up with cold RPMI to 50 mL.

12. Centrifuge the tubes for 90 seconds at 800RPM and 4°C.

13. Pour off the supernatant and repeat this step one more time.

14. Pour off the supernatant and take tubes to the tissue culture hood. Add pen/strep to RPMI 1640 containing 10% FBS to a final concentration of 1% to make the islet culture media. Resuspend the islets in 5 ml of this culture media and put them in a 10-cm petri dish

Note: *This protocol is meant to be a starting point; all isolation procedures require an individual optimization. COL G and COL H concentration, protease addition and digestion time can be experimentally adjusted.*