

**The Queen's Medical Center
Center for Biomedical Research**

STANDARD OPERATING PROCEDURES

TITLE: Isolation and preparation of pancreatic islets and beta-cells		
Original Issue: 11/19/09	Revision Date: none yet	Page 1 of 5
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Personal Protection:

1. Wear lab coat, eye protection and gloves at all times at preparation for, during surgery and clean-up. Observe the following safety plans (available on the QCBR intranet):
 - a) UH Chemical Hygiene Plan (CHP)
 - b) QCBR Biological Agents and Bloodborne Pathogene Exposure Control Plan
 - c) QCBR Waste management plan for biological waste disposal of blood and biological waste.
 - d) Standard Operating Procedures (this document).
2. All employees working at the LCMS must be up-to-date on the CHP/general laboratory safety training offered through Mark Burch, UH chemical safety officer (956-5180).
3. Employees working with animals must have completed the UH WebCT Training "Review of the Regulations for Care and Use of Vertebrate Animals".

Suction Flow Table Preparation:

1. Make sure the main ventilator controlling the suction flow at the surgical tables is turned on.
2. Open the valve control at the table exhaust to start suction flow.
3. Prepare Anesthetic machine.
4. Prepare surgical tools and heat blanket.

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Isolation of Rodent Islets

Preparations before arrival of the animal(s):

Mouse and rat islets are isolated in-house through the following procedure. Plan to do no more animals than surgeries can be finished within 1 hour. Turn-on water bath to 37°C. Buffer solutions must be prepared fresh on the day of isolation. Prepare a solution of collagenase (1.5 mg/ml for rat and 0.8 mg/ml for mouse) and trypsin inhibitor from soybean (1.0 mg/ml) in sterile Hank's Buffered Saline Solution (HBSS). Fill a 10-ml syringe with 8-9 ml of solution with a 27-gauge needle. Attach stiff tubing to 27-gauge needle. Cut the open end of the tubing at an angle to resemble a syringe. Keep on ice.

Animal Surgery Procedures:

For islet isolations, rats should be between 125-300 g and mice between 10-40 g. Animals are anesthetized in a dessicator using enflurane. **THIS REQUIRES TRAINING THROUGH THE VETERINARIAN BEFOREHAND.** Once the animal is anesthetized (as determined by non-response to the pinch method and lack of blink response), it will be taken out of the dessicator and immediately euthanized by cervical dislocation.

The exterior of the abdomen is washed with 70% ethanol. Expose the pancreas by making a V-cut from the lower abdomen up to the rib area. Locate the pancreatic duct and remove any excess fat. Clamp off the pancreatic duct with a hemostat at its duodenal insertion. Using a dissecting microscope and microscissors, cut the duct one-third way across and insert the tubing.

Hold the tubing in the duct and rapidly inject the collagenase/soybean trypsin inhibitor solution. The pancreas should be distended and fully dilated after 6-ml

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injection. After the collagenase infiltration, carefully remove the pancreas. Start from the intestines, moving to the stomach and then the spleen. When the pancreas is only attached by the bile duct, cut it out of the rat. Place the pancreas in an empty 50-ml conical tube. All surgery should be completed within an hour; otherwise the first pancreas will start digesting. Up to two pancreata can be placed into one tube.

Put all pancreas in the water bath at 37°C for 20-22 minutes. At the end of the incubation, add 20 ml RPMI 1640 medium with 10% FBS. The rest of the isolation should be done on ice. Hand-shake the tubes vigorously for 5-10 seconds to break up the tissue. Wash islets 3 times to remove collagenase with RPMI 1640 +10% FBS and centrifuge at 800 rpm for 1 minute. Pour off supernatant. Resuspend in 25 ml of RPMI 1640 +10% FBS and place entire volume into a 150 mm petri dish. Pick islets using sterile 100 µl pipet and dissecting microscope. Avoid picking other tissue. Place islets in 6-well dish with sterile HBSS.

Place all islets into a 50 ml conical tube and centrifuge at 800 rpm for 1 minute. Aspirate supernatant and add 2 ml of 0.25% trypsin-EDTA. Incubate in waterbath for 8 minutes. Add 18 ml RPMI 1640 + 10% FBS. Centrifuge at 800 rpm for 1 minute. Aspirate supernatant and repeat rinse and spin 2 additional times. Resuspend in 1.5 ml RPMI 1640. Using a 1 ml sterile pipet, gently break islets into individual cells. Plate cells by aliquoting 60 µl of the cell suspension onto polylysine coverslips in a 24-well plate. Incubate the cells for 2 hours at 37°C in a humidified incubator (5% CO₂). Following the 2 hour incubation, add 1 ml of RPMI 1640 to each well. Let the cells sit overnight in the incubator before doing experiments.

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General Remarks about Handling of Rodent Islets

Several aspects must be considered before islets are used for single cell experiments. First, islet purity is of utmost importance. If the islet preparation contains more than 20% acinar tissue, it is better not to use the islets for single cell experiments. This significantly lowers the probability of finding b-cells and also leads to growth of fibroblasts on the tissue culture plates which cover the plate by the second to third day of culture. As fibroblasts grow on the tissue culture plates, they grow over the top of the b-cells, blocking the detection of insulin with microelectrodes. A second issue that must be considered is the *in vitro* culture time of the islets. If the islets have been in culture for more than 2 days total (including the shipping time), it is best to use them only for single-islet experiments. Islets become more fragile in culture leading to increased sensitivity to the dispersion enzymes, thereby increasing the likelihood of cell damage. Finally, sterility in handling must be strictly observed. Islets that have been in culture are much more easily contaminated than freshly isolated islets necessitating strict adherence to sterile procedures.

Islets should be checked for sterility, purity and structural integrity. For this purpose, the exterior of the islet container is sterilized with 70% ethanol followed by 70% isopropanol. After the islets are mixed thoroughly by shaking the centrifuge tube in which they are kept, a 3 ml aliquot is removed and transferred to a 35 mm tissue culture dish. The sample is then observed under both a dissecting microscope at 40X magnification and an inverted, compound microscope at 400X (or greater). Observation under the dissecting microscope allows determination of structural integrity and purity. Islets have a golden color (Figure A-1) while acinar tissue will be gray or black. Also, islets are denser and have a spherical or oblate spherical shape while acinar tissue is less

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organized structurally. In many cases, large animal islets will be over-digested, making it more difficult to distinguish between islet tissue and acinar tissue. Experience will help aid in purity determinations. Finally, the sample should be observed under the higher magnification to check for bacterial contamination. Typical bacteria can be seen as 1-2 mm rod shapes that move randomly in tissue culture media, if present. If bacteria are present in this initial stage, islets should be discarded immediately.

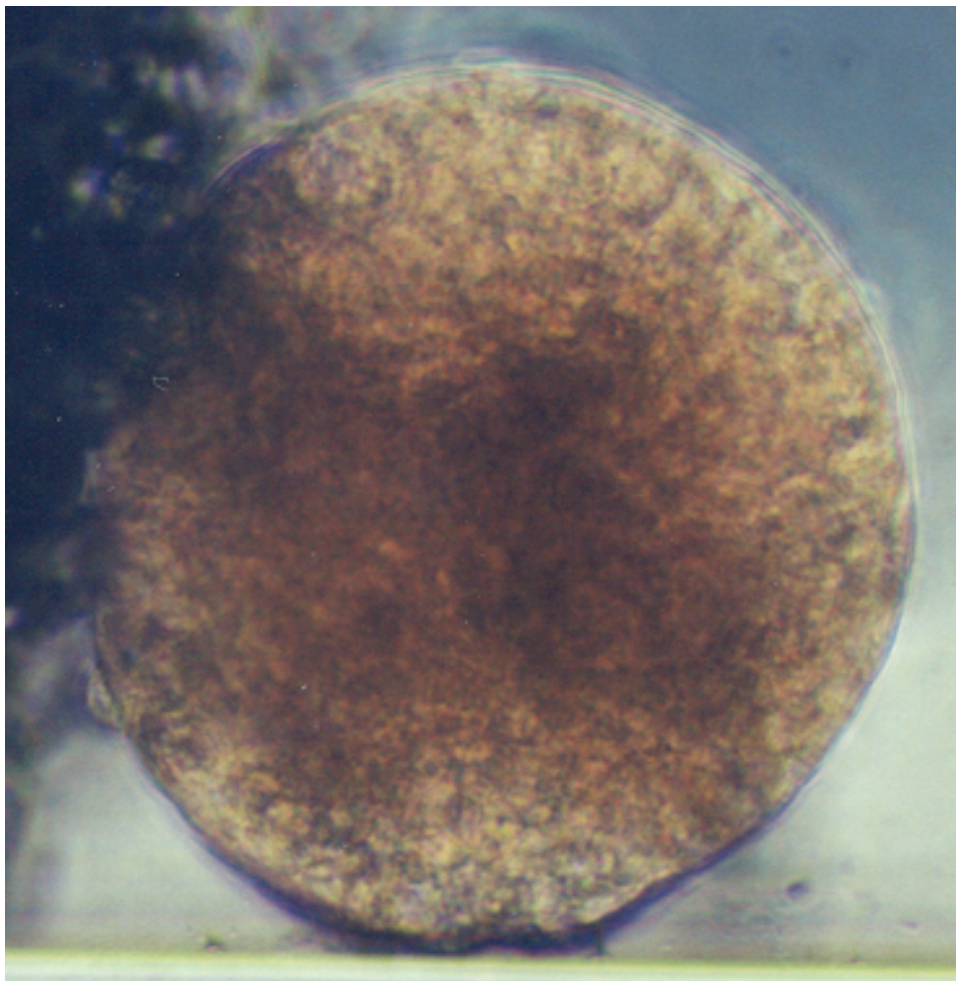


Figure A-1. Typical mouse islet of Langerhans isolated using ductal injection of collagenase. Islet was obtained according to procedure described in text. Glass beads on upper left of islet were used to hold the islet in place and have no significance for the isolation described above. The islet shown is approximately 150 μ m in diameter