

Kidney Capsule -Islet Transplantation Protocol**Materials and Solutions**

Item	Vendor/Cat. #	Item	Vendor/Cat. #
Surgical gloves	Fisher #11-394-95(sz)	Glass syringe	Hamilton #1001
Surgical scrub sponges	Moore #42940AK	Cauterizing tool	Roboz #RS230
Forceps w/ teeth, n=2	Miltex # 6-114	Needle Holder	Moore #41-067
Forceps/straight, n=2	Miltex #6-26	5-0 silk suture (6-C)	Look #754B
Dissecting scissors n=2	Miltex #5-290	Cotton tipped swab	Moore #
Oster razor (sz. 40 blade)	Fisher #01-305-10B	9mm autoclip stapler	BD #7630
4x4 Sterile gauze pads	Moore #08252AK	9mm staples	BD #7631
Povidone Iodine pads	Moore #08486AK	9mm staple remover	BD #7637
Alcohol pads	Fisher #14-819-2	Heating pad	Moore #42508AK
25 G x 1/2" needles		Cidex solution	Moore #07535AK
PE50 polyethylene tubing (0.965mm O.D. x 0.58mm I.D.)	BD # 427411	Cidex + 28 day soln	Moore #35625AK
Silicone tubing (5/32"OD x 1/32" ID)	Spectrum Chromat. #123732	Instrument sterilizing container	Moore #39074AK
Straight Pipet tips, sterile	TipOne #1111-0810	from USA Scientific	

1. Preparation of Islets for Transplant (Tx)

- Hand-pick islets using a P200 pipetman and straight pipet tip (TipOne) under an inverted microscope, from the cultured islets in a 100mm plate.
- Count islet 100 at a time and transfer into each microcentrifuge tube (~500 islets/tube/mouse).
- Allow islets to settle to the bottom of microcentrifuge tube.
- Draw one islet pellet into a P200 pipetman (set at 130ul) and the straight pipet tip.
- Place a silicone tube adapter over the syringe tip. Insert PE50 tubing into the adapter.
- Hang the pipetman to the side of the hood and tape the tubing to the hood wall higher than the islets in the tip. This will allow the islets to settle in the tip in the pipet.
- Transfer the islets into the PE50 tubing by slowly dialing the pipet and moving the islets into the p50 tubing (Being careful not to expel the islets from the end).
- Make a kink in the end of the tubing. While maintaining the kink, disconnect the tubing from the pipetman. Secure the kink with the silicone tube adapter.
- Place the islets in the PE50 tubing, kinked silicone adaptor side down, into a 15 ml conical containing a cut 5mL pipet. Tape end of p50 tubing over 5mL pipet so it does not curl in tube during centrifuging.
- Centrifuge tube to 1000 rpm and turn off centrifuge. (Never prepare more than 10 islet preps. at one time).
- Tubes can be places on ice or stay at room temperature.

2. Preparation of Mouse for Tx

- Anesthetize the mouse using Isofluorane or inject ~100ul *i.p.* (varies w/ size) of Ketamine/Xylexene (see preparation protocol).
- After anesthetic has taken affect, shave the left flank of the mouse.

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- c. Swab skin of mouse, center – out, with Povidone Iodine swab and then wipe off with an ETOH swab.
 - d. Locate the left kidney (just right of spleen). Make a small incision in the skin, exposing the peritoneum.
 - e. Make a small incision in the peritoneum exposing the kidney. (small incisions are important; they help in keeping the kidney raised and exposed)
 - f. By applying slight pressure to both sides of the incision, raise or pop the kidney out of the mouse.
 - g. Using a cotton tipped swab, continually moisten the kidney with sterile saline supplemented with Gentamycin.
 - h. Using a syringe 25 gauge needle, make a small scratch on the right flank of the kidney, creating a nick in the kidney capsule.
3. **Transplantation of Islets** (While the mouse is being prepared for Tx the second person should prepare the islet transplant syringe)
- a. Slowly remove the silicone adaptor from the PE50 tubing while keeping the kink in the tubing. Place the opposite end of the PE50 tubing into the silicone adaptor and place the silicone adaptor onto the tip of the “screw-locked” glass syringe then slowly release the kink. Advance the islets slowly to the tip of the PE50 tubing using the “screw” mechanism.
 - b. Carefully slide the PE50 tubing under the kidney capsule through the small scratch in the capsule, make a small pocket, being very careful not to gouge the kidney or puncture through the capsule.
 - c. It helps to keep the area and capsule moist with a normal saline-Gentamycin soaked cotton tipped swab.
 - d. Gently move the tubing in all directions, creating a “pocket” for the transplanted islets to rest.
 - e. The second person, who prepared the mouse and islet transplant syringe, will slowly advance islets into “pocket” until all islets are transferred, under the direction of the person who has opened the mouse, exposed the kidney, and is currently directing the PE50 tubing under the capsule.
 - f. Remove the PE50 tubing slowly, dry the area with a dry swab and carefully cauterize the nick.
 - g. Using a dry cotton tipped swab make sure all bleeding has stopped. Once bleeding has stopped re-moisten the kidney with sterile saline, gently replace the kidney into the peritoneum, prior to closing the mouse.
4. **Closing/Revival of Mouse**
- a. Close the peritoneum with a running stitch using 5-0 silk sutures w/ a C-6 19mm needle.
 - b. Using forceps draw both side of the skin incision together.
 - c. Then staple the skin together with 2 or 3 staples.
 - d. Clean the skin of the mouse, of any blood using a cotton tipped swab and saline.
 - e. Place the mouse in a cage, which is sitting on a heating pad or below a heating lamp, until the mouse is fully active.
 - f. Remove the skin staples in 2 weeks.