

Isakson Lab Method 01/05/08

The Carotid Exposure Surgery Method

F-127 pluronic gel stock prep:

When preparing the 50% pluronic gel stock, weigh out 10g of solid F-127 pluronic acid into a 50mL centrifuge tube. Next, lay the tube on its side so that the solid spreads out along the surface. Finally, add 20mL of sterile filtered water to the tube while keeping it slightly tipped so that the solid remains on the side. Allow solid to dissolve on a rotator at 4°C, this should take about 24-48 hours. When using the gel on a daily basis, transfer a few mL from the stock tube into another sterile tube each day so as not to repeatedly pipette from the sterile stock.

Lipid stock preps:

A good working stock is 1mg/mL. Dilute in chloroform for easier evaporation later.

F-127 gel with added Lipids:

Add specified amount of lipid of interest to a glass culture tube and evaporate the liquid using argon while placing the tube on a vortexer. This keeps the lipid from forming a pellet at the bottom of the tube. Add specified amount of F-127 pluronic gel to the tube and vortex for about 2 minutes. The best way to do this is to alternate between vortexing and placing the tube on ice to keep the gel in the liquid state. I do 15 seconds on the vortexer, then 15 seconds on ice until I reach a total of 2 minutes on the vortexer. Keep tubes on ice the entire time that you are using them.

Surgical procedure:

- 1) Give mouse an IP injection of 80:8 mg/kg ketamine/xylazine solution (usually around 100uL per mouse) and allow mouse to reach an acceptable plane of anesthesia. This is achieved when the mouse no longer has a foot withdrawal response to foot pinch (its whiskers usually aren't moving anymore either). Don't be confused by normal deep tendon reflexes which you will elicit if you pinch the foot too hard.

- 2) Give mouse a SC injection (in the back) of a 1:10 dilution of buprenorphine in sterile water (around 80-100uL).
- 3) Mark mouse's tail using surgical marker for easier identification later.
- 4) Apply ophthalmic ointment to mouse's eyes.
- 5) **Very carefully** shave mouse's anterior neck with a scalpel and using ethanol to wet the fur. The best method that I have found is to lift the skin and only shave parts of the skin that have been lifted away from underlying organs. I personally have compromised the mouse's trachea several times by not lifting the skin, so I strongly suggest you adopt this method or find another one to avoid crushing its trachea during shaving.
- 6) Carefully tape (can also use adhesive drapes) mouse to a sterile working surface. I use the abdominal pads as they are both sterile and absorbent in case anything spills.
- 7) Wash anterior neck area with 75% ethanol followed by iodine solution.
- 8) Don appropriate sterile gloves and mask.
- 9) Lift skin in the midline just superior to the clavicle and cut away a small chunk with scissors.
- 10) Continue incision with scissors upward towards mouse's chin.
- 11) Using jeweler's forceps, carefully separate the two lobes of the thymus and begin to work down to the left common carotid artery. I find that the best way to stabilize the forceps is to hold them between the index finger and thumb with the bulk of the instrument resting on the top surface of the hand.
- 12) Carefully remove tissue above the carotid artery with the left forceps while using the right forceps to hold tissue out of the way, thus creating an opening in the mouse's neck area.
- 13) The carotid artery will be the large one pulsating just lateral to the trachea.
- 14) Carefully free the carotid from the muscle and tissue around the trachea and begin to dissect it.
- 15) Dissect the carotid by carefully removing overlying tissue and by opening forceps along the surfaces of the vessel to strip tissue away. If possible, separate the carotid from the vagus nerve as this will further remove connective tissue around

- the vessel. (Note: the vagus nerve is the white, stringy object directly lateral to the carotid)
- 16) Using the right-hand forceps, carefully put the tip under the carotid, open the forceps, and lift the carotid slightly away from surrounding tissue.
 - 17) While holding carotid in place, use your left hand to pipette pluronic gel. First cool pipette tip by rinsing several times in cold sterile water. Next eject all water and place tip into gel and withdraw 60uL. Wait a few seconds for gel to equilibrate in pipette tip.
 - 18) While still holding carotid away from surrounding tissues, inject the 60uL of pluronic gel in the milieu of the vessel. The gel will begin to solidify to a toothpaste-like consistency and should fully surround the vessel.
 - 19) Carefully remove the right-hand forceps from under the vessel and allow it to rest in the gel.
 - 20) Place the lobes of the thymus back to original position and use the left lobe to maintain the pluronic gel in the area that you dissected.
 - 21) Pull skin together and close wound using a continuous suture. (Note: DO NOT use a continuous suture if you are doing survivals for longer than a few days as the mice will eventually figure out how to chew the sutures out. If doing longer survival times, use the interrupted suturing method.)
 - 22) Give mouse a SC injection (in the back) of 1mL of sterile saline warmed to 39°C in a water bath.
 - 23) Check mouse's eyes to ensure that they are still sufficiently covered with ointment.
 - 24) Allow mouse to recover under a heat lamp until he begins to try to crawl again.
 - 25) Place mouse back in original cage and continue onward with your surgical ventures.

Perfusion Fixation:

After your specified survival time, you will need to perfuse the mouse in order to adequately preserve the carotid artery. Prior to beginning, make sure that you have

adequate amounts of 2% paraformaldehyde (PFA) and 1X PBS. I like to have at least 500mL of 2% PFA available for perfusion and tissue soaking.

- 1) Sacrifice mouse via CO₂ asphyxiation.
- 2) Pin mouse to an absorbent pad as this procedure is quite messy.
- 3) Make an incision in the mouse's abdomen and continue upward towards your previous surgical area.
- 4) Fold skin and overlying tissue aside and carefully dissect away the diaphragm to access the thoracic cavity.
- 5) Carefully cut away and remove most of the mouse's ribcage while ensuring that you do not puncture the heart.
- 6) Once the heart is exposed fully, grasp it with blunt tongs and puncture the left ventricle with an IV catheter. Guide the catheter tip into the ventricle a bit and then withdraw the needle. It is not necessary to activate the needle safety device as the needle can be used for multiple mice. Clamp the catheter into place in the left ventricle using a very small clamp. (Eventually you may want to attempt to insert the catheter into the aorta to ensure more complete perfusion, but this is not at all necessary and can sometimes do more harm than good.)
- 7) Attach 1X PBS line to IV catheter and open line to begin perfusion.
- 8) Cut a slit in the right atrium to allow fluid to make a complete circuit through the systemic circulation and then leave the body. If you do not see fluid running out of the right atrium, check to make sure that you are not perfusing the pulmonary circulation which will be evidenced by big, white lungs and fluid coming out of the mouse's nose. If this happens, carefully redirect the catheter into the left ventricle.
- 9) While perfusion is occurring, dissect out the surgical area and expose the carotid artery.
- 10) Allow the mouse to perfuse with 1X PBS until the liver has mostly lost its color and the fluid running out of the right atrium begins to look less bloody. At this point, the body is largely purged of blood.
- 11) Stop flow of 1X PBS and attach line for 2% PFA to catheter. Open line to allow PFA to flow and begin fixation.

- 12) As PFA is perfusing, drip several mL of PFA onto the outer surface of the carotid artery.
- 13) Allow perfusion with PFA to occur for somewhere in the 3-5 minute vicinity and then prepare to quickly remove the carotid artery.
- 14) Make a quick surgical knot around the inferior aspect of the carotid artery to give orientation later on.
- 15) Carefully and quickly remove the carotid artery from the mouse. If you get some surrounding tissue, it is okay, but try to keep it to a minimum for easier identification later on.
- 16) Place the dissected carotid artery into a tissue cassette and soak the cassette in a jar of 2% PFA for one hour.
- 17) At the end of the PFA soak, move the cassette into 70% ethanol for storage until delivery to research histology core.
- 18) Place the mouse's body inside of a pair of gloves and freeze it for later disposal.