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 We use this protocol and it's working

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Abdominal Emitter Implantation

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MATERIALS

Surgery Preparation:

Standard G2 Emitter (Starr Life Sciences Corp)

Sterile 1x PBS (pH =7.4)

Glutaraldehyde plus Sterilizing and disinfecting solution and Activator plus (Regimen)

Three (3) 50ml sterile Falcon tubes and holding rack

Label them for glutaraldehyde and two PBS washes

Note: Emitter calibration is performed prior to implantation according to Starr Life Sciences Corp's protocol using according receiver and software. Serial numbers for each implanted emitter (printed on each emitter) needs to be recorded per mouse **prior** to implantation.

Surgery Check list:

Tissue forceps (sterile)

Large tweezers (at least 20cm long) (sterile)

#10 Stainless steel blade (Bard-Parker – sterile)

5-0 PDS suture (sterile)

Super glue (Loctite 454)

Gauze (sterile)

Cotton swabs (sterile)

Nolvasan

70% ethanol

Two (2) 50ml sterile Falcon tubes in a tube rack

Label them "Alcohol," "Nolvasan"

Tube rack with your glutaraldehyde and two PBS wash falcon tubes with your Emitters inside your

glutaraldehyde tube for at least 24 hours

Electric hair trimmers

Three (3) surgical towels (sterile)
Heating pad
Glass bead sterilizer
Kimtech paper
Eye lubricant
Surgical suite and animal housing
Surgery cards and surgery record
Personal protective equipment (PPE): lab coat, gloves, face mask
Isoflurane vaporizer, oxygen tanks, and anesthesia induction chamber with nose cone attachment
Carprofen
Sterile Saline
0.5 mL syringes (for carprofen)
3 mL syringes (for saline)
70% ethanol spray

Surgery Preparation

- 1 Preparation for implantation of Emitters has to occur at least 24 hours prior of the surgery time for proper sterilization of the Emitter.
- 2 In your 50ml falcon tube mix 25ml Glutaraldehyde plus solution and 800ul Activator plus solution (=Glutaraldehyde Sterilization Solution). Vortex the falcon tube until solution is adequately mixed.
- 3 Gently place Emitters into the Glutaraldehyde Sterilization Solution. Make sure every Emitter is completely submerged. Incubate for at least 24 hours and up until the implantation surgery for proper sterilization.
- 4 Prepare two Falcon tubes with approximately 40ml sterile 1x PBS to clean off all Glutaraldehyde Sterilization Solution right before implantation.

Surgery

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- 6 Fill your "Alcohol" tube half way with 70% ethanol, "Novalsan" half way with Novalsan solution. Place sterile cotton swabs inside both of the tubes with the cotton tip submerged in both of the solutions.
- 7 Place your sterile towel on top of your heating pad and turn it on to approximately 100°F (~37°C). Place the nose cone connected to the isoflurane vaporizer on top of the towel.
- 8 Place another sterile surgical towel down next to your heating pad and place your sterile tools, wound clips, wound clip applicator and gauze on top of it. Place your two tube racks (one with alcohol and chlorohexidine and the other with your sterile emitters and PBS tubes) next to the towel.
- 9 Record the mouse's body weight to prepare syringes for saline and analgesia treatment. Warm saline (1-1.5 mL for 25 g body weight); carprofen (5-10 mg/kg body weight.)
- 10 Induce anesthesia with 5% isoflurane in induction chamber.
- 11 Place the mouse on the surgery platform and maintain anesthesia via a nose cone at 1.5-2% Isoflurane/oxygen (adjust for each mouse accordingly to breathing and anesthetic depth (e.g. loss of withdrawal reflex)).
- 12 Apply eye lubricant to the mouse's eyes.
- 13 Disinfect the lower back of the mouse with 70% ethanol, then administer subcutaneous carprofen injection in the disinfected area.

- 14** Check for full withdrawal reflex to ensure anesthetic depth.
- 15** Place the mouse into dorsal recumbence.
- 16** Use clippers to shave the abdomen of the mouse.
- 17** Disinfect the shaved skin by three alternating scrubs of Novalsan followed by 70% Isopropyl rubbing alcohol.
- 18** Make a 1cm ventral midline incision with your #10 blade through the skin and subcutaneous layers.
- 19** Make a reverse stab incision through the linea alba using the #10 blade
- 20** Using your large tweezers, remove emitters from the Glutaraldehyde Solution and place into sterile PBS, swirl to remove Glutaraldehyde Solution (~30 seconds). Move the emitter to the 2nd tube with PBS and swirl again (~30 seconds). Make sure to keep tubes closed to prevent contaminations. Take out the emitter with your large tweezers and place it on sterile gauze.
- 21** Using tissue forceps, grab the emitter and insert it into the ventral midline incision caudal to the left kidney
- 22** Close the muscle layer with 5-0 PDS in a cruciate patter then close the skin using 5-0 PDS in a simple interrupted and cruciate pattern. Apply super glue over the suture.

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- 23** Remove the mouse from the nose cone and administer intraperitoneal injection of warm saline for rehydration
 - 24** Place the unconscious mouse in a fresh cage with one cage side on a heating mat and monitor until startling reflexes are restored.
 - 25** Replenish all consumables (sterile cotton swabs, sterile gauze) and use 70% ethanol to clean surgical tools and place in glass bead sterilizer for 15-20 seconds before additional surgeries.