



# MMPC Surgical Protocol

Version: 1

Replaced by version: N/A

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## Summary:

Study of the unstressed mouse requires prior implantation of catheters in the carotid artery and jugular vein. The arterial catheter is used to sample blood and the venous catheter is used as an infusion port. The surgical procedure is performed under sterile conditions in accordance with AAALAC standards.

## Reagents and Materials:

### Tubing

**Stainless steel, 25ga.** Ziggy's Tubes and Wires # 25R304-36

**Wire, stainless steel.** Ziggy's Tubes and Wires # W020304V-1

**Silastic tubing 0.012"i.d.** Fisher Scientific # 11-189-14

**Silastic tubing 0.020"i.d.** Fisher Scientific # 11-189-15A

**Silastic tubing 0.058"i.d.** Fisher Scientific # 11-189-15E

**Polyethylene tubing (PE-10).** Fisher Scientific # 14-170-12P

**Polyethylene tubing (PE-20).** Instech Solomon # BPE-T20

### Surgical Tools

**Forceps, Dumont 7b curved shank.** Fine Science Tools # 11270-20

**Hemostats.** Fine Science Tools #13020-12 (smooth), 13010-12 (serrated)

**Micro-serrefines.** Fine Science Tools # 18055-03 (straight); 18055-05 (curved)

**Needle holder.** Fine Science Tools # 12060-01

**Spring scissors.** Fine Science Tools # 15003-08

**Scissors, surgical.** Fine Science Tools #14028-10

**Forceps.** Roboz # RS-5136. RS-5110. RS-5111

**7-0 nylon suture.** Any preferred vendor.

**6-0 silk suture.** Any preferred vendor.

**14-gauge needle, dulled**

### Additional Materials

**Clippers.** Oster MiniMax trimmer. Patterson Veterinary Supply #07-842-4245

**Nolvasan Surgical Scrub.** Fort Dodge Animal Health NDC 0856-1041-03

**70% Alcohol.** Any vendor.

**Isoflurane, USP Liquid for Inhalation.** Piramal Healthcare, NDC 66794-017-25

**Ketofen.** Fort Dodge Animal Health, NDC 0856-4396-01.

**Saline, Bacteriostatic 0.9% Injection USP,** 30mL btl. Hospira, Inc. NDC 0409-1966-07

**Heparin, Sodium Injection, USP.** 1000U/mL. Hospira, Inc. NDC 0409-2720-02

**Ampicillin** for injection, USP, 250mg . APP Pharmaceuticals, LLC NDC 63323-387-10

**Silicone glue.** Medical Adhesive Silicone Type A. Dow Corning # 891

# Protocol:

## Preparation:

1. Anesthetize mouse. Bevel catheters to correct lengths\*, fill with 200U heparinized saline and plug.
2. Surgery must be conducted in a disinfected area that promotes asepsis.
3. Prepare the animal by removing hair from the surgical site. Perform this procedure in an area separate from where the surgery is to be conducted.
4. Prepare the surgical sites with an appropriate skin disinfectant (Nolvasan followed by alcohol).
5. Administer Ketoprofen, 5-10 mg/kg subcutaneously.
6. Surgeons wash and dry their hands before aseptically donning sterile surgical gloves.

## Surgery:

1. Make small vertical midline incision 5 mm cephalic to the xiphoid process.
2. Blunt dissect using forceps to expose the left sternomastoid muscle. Reflect this muscle to expose left carotid artery.
3. Gently tease off connective tissue from the carotid artery. It is important at this point to isolate the vagus nerve from the artery without damaging either the artery or the nerve. Isolate artery then ligate cephalic end with silk suture. Another piece of suture is loosely knotted on the caudal end of the exposed artery.
4. Clamp vessel with micro-serrefine and cut just below the ligated end with spring scissors. Carefully insert catheter as far as possible. Grab catheter with forceps, then carefully release micro-serrefine clamp and continue inserting catheter to the silastic-polyethylene junction. At this point the tip of the catheter should be in the aortic arch.
5. Tie both ligatures securely and confirm that the catheter samples. Flush with heparinized saline.
6. Make another incision 5 mm to the right of midline and about 2 mm caudal to the first incision.
7. Blunt dissect to expose right jugular vein.
8. Isolate carefully and ligate cephalic end with silk suture. Loosely tie another piece of suture at the caudal end of the exposed vein.
9. Cut just below cephalic ligature with spring scissors. Insert catheter to the bead, tie and confirm that it samples. Flush with heparinized saline.
10. Turn mouse over. Make a small incision between the shoulder blades. Tunnel 14-gauge needle under skin through the incision on the back. Thread catheters through the needle to exteriorize them to the back of the mouse.
11. Close ventral incisions with nylon suture.
12. Clamp venous catheter with micro-serrefine at the incision site between shoulder blades. Cut catheter 1 cm above clamp and connect to \*\*MASA<sub>tm</sub>. Take care to ensure that there are no holes or kinks in the catheter. Secure venous catheter to MASA<sub>tm</sub> with silk suture. Repeat for the arterial catheter.

13. Close dorsal incision with nylon and confirm patency of both catheters again. Flush with heparinized saline. Place mouse in warmed, clean cage.

**\*Arterial Catheter Preparation:**

1. Cut 10 cm of PE-10 and carefully stretch the tubing to ~13 cm. If the tubing is stretched much beyond this point it will snap.
2. Cut stretched PE-10 into 1.3 cm pieces.
3. Cut silastic tubing (0.012"ID) into 6 cm pieces.
4. Insert Dumont forceps into the end of a 6 cm piece of silastic. Gently open forceps so the tubing is opened wide.
5. Gently grasp the end of a stretched and pre-cut piece of PE-10 using a pair of Graefe forceps, and insert the tip of the PE-10 into the silastic tubing approximately 3 mm.
6. Slip the Dumont forceps from the silastic tubing, leaving the PE-10 in place. The catheter tip is beveled at the time of surgery.

**\*Venous Catheter Preparation:**

1. Cut a 6 cm piece of silastic tubing (0.012" ID).
2. Cut a 1 mm section of silastic tubing (0.020" ID) for use as restraining bead.
3. Insert the tips of eye dressing forceps into the lumen of the restraining bead and gently hold the tips of the forceps apart to stretch the opening wider.
4. Using another pair of eye dressing forceps, slide the silastic tubing into the lumen of the restraining bead.
5. Pull the silastic tubing through the bead until it reaches 11 mm.
6. The bead must lie flat around the catheter.
7. Adjust the bead and bevel the catheter tip at the time of surgery making any necessary adjustments for differences in mouse size.

**\*\*MASA<sub>tm</sub> Preparation:**

1. Cut two 1.3cm long stainless steel tubes using file, smoothing edges with abrasive stone.
2. Cut two 2.5cm long PE-20. Attach #2 to #1.
3. Bend each stainless steel tube in half at a 120° angle.
4. Slide silastic tubing (0.058" ID) over where both ss tubes and PE-20 is attached.
5. Place completed rig in silicone medical adhesive for 24 hours.
6. Catheter plugs for MASAtm are cut from steel wire for use at time of surgery.

## **Reagent Preparation:**

### **Reagent 1:**

#### **200U Heparinized Saline with 5mg/mL Ampicillin**

#### **Reagents and Materials**

**Saline, Bacteriostatic 0.9% Injection USP, 30mL btl.** Hospira, Inc. NDC 0409-1966-07

**Heparin, Sodium Injection, USP.** 1000U/mL btl. Hospira, Inc. NDC 0409-2720-02

**Ampicillin** for injection, USP, 250mg . APP Pharmaceuticals, LLC NDC 63323-387-1

#### **Procedure**

Reconstitute Ampicillin. (Follow package insert directions).

Withdraw 6 mls from 30ml btl of saline.

Add 6mls of Heparin.

Add 0.5mL of reconstituted Ampicillin.

Keep in fridge, discard after 1 week.