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## Subrenal capsule (SRC) implantation of tumor tissue

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol describes the steps required for the successful implantation of tumor tissues under the subrenal capsule. The subrenal capsule is an optimum site for the propagation of tumor tissue as it provides an environment rich with blood vessels and growth factors.

## Guidelines

Species: Mouse, Male.

Strain: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ

Age: 6-8 weeks.

Vender: Jackson Laboratory

Housing: House animals within the university-specific IACUC approved housing mouse colony facility at a weight ranging 14-19 grams.



## Materials

	Material	Source	Catalog Number
	Wound clipper set	Sai Infusion	ACKIT9MM
	Surgical Instruments	Kent Scientific	INMOUSEKIT
	Sterile cotton tipped applicators	Puritan	25-806 10WC
	Povidone-Iodine Prep Pads	MEDLINE	MDS093918
	Phosphate buffered saline	Gibco	14040117
	Petri dishes	Millipore Sigma	P5481
	Pasteur pipette	Corning	7095D-9
	Ophthalmic ointment	Akorn	59399-162-35
	Lidocaine Hydrochloride 2% w/v Solution	ADVANZ Pharma	N01BB02
	Ice bucket	Corning	1167U68
	Disposable sterile pads	Medline	MSC281224
	Disposable scalpels	Exel international	29550
	Buprenorphine	Covetrus	059122
	Alcohol Prep Pads	WEBCOL	6818
	Absorbable sutures	CP Medical	421A – VISORB® 5/0 FS-2 30"
	70% Ethanol	Carolina	861261

	Material	Source	Catalog Number
	1 cc syringe	BD	309628
	Isoflurane	Piramal Critical Care(RxElite )	66794001725
	HEPES- buffered saline (HBS)	Gibco	15630080

## Equipment

### 1. Isoflurane small animal anesthesia machine



Small animal anesthesia system

### 2. Water circulating system

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### 3. Heating pad/Circulating water blanket/pad



### 4. Weighing Scale



5. Bead Sterilizer

6. Bain breathing tube

7. Mouse nose cone

8. Mouse knock down box

9. Nair or any hair removal product/instrument

## Troubleshooting

## Preparation before surgery

- 1 **Fire bent glass pipette:** Prepare the bent pipette by exposing the pipette tip to fire and slightly bending it into an inverted C shape with the help of forceps. The image below shows an example of bent pipettes.



Note: Make sure there are no sharp edges on the pipette tip as that will cause a tear in the renal capsule.

- 2 **Preparation of surgical instruments and supplies:** All of the instruments and supplies should be sterilized.
- 3 Surgery record sheets

	A	B	C	D
PI				
Personnel				
Date				
Procedure name	Sub-renal implantation			
Protocol #				
Type	Survival Surgery			
Species	Mouse			
Experimental agents administered	Tumor tissue			




	A	B	C	D
	<b>Anesthetics</b>	Isoflurane	Dosage: (1-5 % or mg/kg; mL)	Route: Inhalation
	<b>Analgesics</b>	(1) Lidocaine	Dosage	Route:
		(2) Buprenorphine	Dosage	Route:

	A	B	C
	<b>Mouse ID</b>		
	<b>Mouse weight</b>		
	<b>Anesthesia start time</b>		
	<b>Analgesics</b>	<b>(1) Lidocaine</b>	<b>Time administered:</b>
		<b>(2) Buprenorphine</b>	<b>Time administered:</b>
	<b>Tumor tissue implantation time</b>		
	<b>Anesthesia end time</b>		

#### Note

This is a template for surgery records and every user should use the template according to their institute's IACUC regulations.

## 4 Preparation of the PDX tissues for implantation from fresh tissue


4.1 Prepare an ice bucket with ice and a petri dish with  10 mL HBS.

4.2 Euthanize the tumor-bearing mouse and extract the tumor.

4.3 Blot the tumor on a Kim-wipe to get rid of excess blood.

4.4 Weigh the tumor and note the weight.

4.5 Using a dissecting scissor, cut the tumor tissue into small sections approximately 1 mm in diameter.

4.6 Place the tissue sections in a petri dish containing  10 mL HBS.

#### Note

The tissues are now ready for inserting into the sub-renal capsule.

## 5 Preparation of the PDX tissues for implantation from frozen biobank

5.1 A cryovial containing frozen tumor sections/slices should be placed on ice to thaw before the surgery.

#### Note

Keep the vial on the ice at all times.

5.2 Transfer the contents of the cryovial into a petri dish containing fresh HBS.



### Note

The tissues are now ready for inserting into the sub-renal capsule.

## Preparation of surgical space

### 6 *Station 1: Fur removal*



Station 1, Fur removal station with anesthesia tubing and nose cone, clippers for hair removal, sterile cotton tips for removal of left-over fur, and kimwipe for cleaning.

6.1 Place a disposable sterile pad on a heating pad.

6.2 Aseptically sterilize the surgical area by spraying with 70% ethanol.

### 7 *Station 2: Surgery*



Station 2, Surgery station with 1) sterilized surgery instruments, 2) sterile dissolvable sutures, 3) lidocaine (0.5%) and 4) buprenorphine, 5) 70% ethanol prep pad, 6) povidone-iodine prep pads, 7) sterile cotton tipped applicators, 8) disposable sterile pad, 9) eye ointment, 10) 1 cc syringe, 11) sterile PBS, 12) ice box with ice, 13) tumor tissue in a sterile petri dish containing sterile PBS, 14) weighing scale, 15) kimwipes, 16) bead sterilizer and 17) nose cone and bair tubing.

- 7.1 Place a disposable sterile pad on a heating pad.
- 7.2 Aseptically sterilize the surgical area by spraying with 70% ethanol.
- 7.3 Place all the autoclaved surgical instruments within the sterilized surgical area.
- 8 **Heat:** Animal should be kept on a heating pad or circulating water blanket/pad during the entire procedure and, after the surgery is over for approx. 2-4 hrs.

## Anesthetization and fur removal of mouse

- 9 Weigh the animal and note the weight.

- 10 Place the animal in a knock-down box circulating with a gas mixture of Isoflurane @ 1.4-2.0% and O<sub>2</sub> @ 1-1.2 lt/min inhalant, maintained via a bain-closed system. Note the start time of anesthesia.
- 11 Move the animal to the station 1 nose cone and apply ophthalmic ointment on the animal's eyes to prevent them from drying out during the procedure.
- 12 Determine the anesthetic depth by pinching the animal's foot for a reflex response.
- 13 Depilate the left dorsal side of the mouse starting from the rib cage till about 4 cm below.

## Surgical procedure

- 14 Move the mouse to the station 2 nose cone and place it on its abdomen.
- 15 Disinfect the surgical area on the mouse with betadine scrub, rubbing in a circular motion. Apply 70% alcohol pads to the surgical area to remove the betadine and loose hair. Repeat this process 2 or more times.
- 16 Make a vertical incision using the straight scissors through the skin along the animal's top curve of the spine (i.e., ~ 0.5 cm below the rib cage) towards the left side of the spine
- 17 Turn the animal so that is laying on its right side.
- 18 Place right thumb on the animal's abdomen, applying very light pressure, and with the left hand pull the skin down, so that the kidney is in view. Once the kidney is located, make a 0.5 cm incision horizontally on the animal's left side with surgical scissors.
- 19 With the aid of the thumbs apply a little pressure on the abdomen to expose the kidney and pop it out of the body cavity. Place the kidney such that it is held by the abdominal wall.
- 19.1 If the incision is made too large, then the kidney will not stay popped out. This incision is critical to the success of this procedure. Do not try to pull on the kidney with forceps as it will cause damage to the kidney and renal vessels





Mouse kept on its right side with left kidney pulled out of the body cavity and ready for incision

- 20 Prevent the kidney from drying out by applying sterile saline using the 1 cc syringe.
- 21 Make a small tear with dissecting scissors in the caudal-lateral aspect of the kidney capsule. With your left hand, hold and gently pull up the capsule layer using forceps. With the right hand, place the insert the bent glass pipette under the kidney capsule layer and glide it from side to side in order to make space for the tissue slice. (Note: Be careful not to puncture the kidney or tear the capsule).
- 22 Use tweezers to place the tumor tissue under the kidney capsule. Advance the tissue slice towards the cranial pole, so that it stays in place and does not leak out once the kidney is placed back in the body cavity.



Mouse kidney implanted with tumor tissue under the capsule



- 23 Return the kidney to the abdominal cavity by lifting the surrounding muscle layer with forceps and letting the kidney slide back into the body cavity.
- 24 Once the kidney is in the body cavity, suture the muscle layer, using absorbable sutures. Pull the skin layer upward and clamp the skin together using a sterile wound clipper, making sure that all of the skin is closed together, and no gaps are left.
- 25 Upon completion of the surgery administer buprenorphine and lidocaine subcutaneously in the right flank. Note the time of administration

#### Note

The concentration of lidocaine and buprenorphine should be kept as mentioned in the institute's IACUC protocol. For this protocol, 0.5% v/v solution of lidocaine was administered.

- 26 Sterilize the surgical instruments using the bead sterilizer by placing them inside for ~ 10 secs.
- 27 Start prepping the next mouse for surgery.

## Post-Op care and monitoring

- 28 Place the animal in a clean cage on a warm heating pad to aid in regaining its body temperature.
- 29 Observe the animal until it has regained full consciousness and is walking around in the cage.
- 30 Remove the clips using the clip remover one week from the surgery
- 31 Observe the animal on a daily basis until sacrificed