

	SOP-BCR-6.7a	Tumor implantation into mice- Tumor Chunk Implant	Author: S. Clouthier  Approved: M. Wicha 	Rev: 2	Issued: 08/14/12 Revised: 09/24/12 Kaitlin Harvey Research Specialist Revised 5/8/2017
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1. Purpose

1.1.1. The purpose of SOP 6.7a is to provide information on proper procedure for implanting tumor pieces into rodent's fat pads.

2. Scope

2.1.1. SOP 6.7a is intended to cover all resources, personnel and equipment in the BCR laboratory.

3. Materials

No	Name	Description	Location
3.1	3% FBS in HBSS		Necropsy Refrigerator (already mixed) Walk-in cooler + Freezer 2 (unmixed)
3.2	Petri Dish	100mm dish for cutting tumor. Or 24 well plate to transplant later	
3.3	Scalpel Blades	#10 blade with blue handle	Necropsy room; top drawer left of hood
3.4	2mL Eppendorf Tube	Autoclaved in Nalgene container in (026-320A-S)	Storage (autoclave prep area)
3.5	BD Matrigel	Basement Membrane Matrix, cat# 354234	-20 Freezer #2 (026-328S)
3.6	1mL Syringe w/ 18g needle	Slip Tip Syringe/ 10b Needle; pink needle hub	Necropsy room
3.7	Surgery Set-up	Micro Surgical Scissor, mouse tooth forceps, tissue forceps, clip applicator and surgery box	Necropsy room- Tools in right drawer sink side. Surgery box on mouse cart
3.8	Isoflurane Nebulizer	Check Iso and O ₂ levels before and during surgery. Notify Kaitlin for help replacing tanks for refilling Iso	Necropsy Room - Left side of hood. Iso stored above sink. O ₂ tanks in cylinder closet 372C

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4. Procedure

- 4.1. Procure tumor from Pathology or from previous implantation from mouse for secondary implantation, or from frozen sample.
- 4.2. A.) **For fresh tumors:** Cut sample into 2mm³ pieces in sterile conditions (do not mince; cross cut the tumor using two scalpel blades making as few cuts as possible to avoid cell damage).
- B.) **For Frozen Tumor Pieces:** Rapidly thaw in 37° C water bath and put directly into sterile petri dish with a ~1mL HBSS with 3%FBS to wash. Carefully aspirate liquid from dish leaving the tumor pieces.
- 4.3. Transfer tumor pieces to a 2mL Eppendorf tube and add 100uL of thawed Matrigel for each tumor piece.
 - 4.3.1. **To implant next day;** choose the fragments to be implanted and place them each into a separate well of a 24-well plate with .5 mL of cell media and place the plate into 37C°/5% CO₂ incubator until implanted.
- 4.4. 1-2 hours before surgery prep mouse by administering analgesic (Carprofen) removing the fur at the surgery site.
 - 4.4.1. Inject 200ul .5mg/mL Carprofen in sterile saline subcutaneously.
 - 4.4.2. Remove abdominal fur from mouse by applying Nair working it into the fur for ~10 seconds and wiping with a damp gauze pad.
- 4.5. Anesthetize mouse via isoflurane inhalation, (Wicha Lab Nebulizer is stored in 374C)
- 4.6. Disinfect surgical site by alternating betadine and 70% Ethanol 3 times.
- 4.7. Using mouse tooth forceps and micro-surgical scissors; make an incision starting slightly below the sternum; cutting along the midline until ~5mm above the genitalia. From the inferior end of that incision make two lateral cuts about halfway down the hind limbs of the mouse making an upside-down 'Y' shape.
- 4.8. Gently separate the skin from the peritoneum using mouse tooth forceps and small fine point sterile cotton swab; exposing the 4th and 5th mammary fat pads of the mouse. Use a sterile 26g needle to pin the skin flap to surgical board.
- 4.9. Using the 18 gage needle attached to 1 mL syringe, draw up tumor pieces suspended in matrigel. If the tumor pieces are too big to draw into needle; use the needle and syringe to suction the piece out.
 - 4.9.1. For the larger pieces that aren't drawn into the needle, make a tiny cut in the fat pad with small surgical scissors, and push tumor pieces into opening with needle. Pinch tissue forceps around needle and draw needle out to keep the piece in the fat pa when the needle is removed.
- 4.10. Close mouse using 9mm surgical slips and place in recover cage. Mice come out of Iso anesthesia within a 1-2 minutes.
- 4.11. Check clips and mouse health daily until clips are removed from mouse; sign off on Surgery Monitoring sheet in animal room.

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5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
6.1	5/8/2016	KH	Revised all. Changed anesthesia to Iso Inhalation only. Update supply list and location.

 <p>University of Michigan Comprehensive Cancer Center</p>	SOP-BCR- 6.7a	Tumor implantation into mice- Tumor Chunk Implant	Author: S. Clouthier  Approved: M. Wicha 	Rev: 2	Issued: 08/14/12 Revised: 09/24/12 Kaitlin Harvey Research Specialist Revised 5/8/2017
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