

Protocols for human breast cancer patient-derived xenograft (PDX) (09/23)

(*Ensure all personnel, mice, materials, etc. are approved by IACUC prior to beginning mouse work)

Materials required

1. Sterile surgery kit
2. Sterile surgery space
3. Sterile razor blades
4. Sterile DMEM/F12
5. 70% EtOH
6. Povidone iodine surgical scrub solution
7. Sutures, staples etc.
8. High quality Basement Membrane Extract such as Matrigel (Corning)
9. Anesthesia (e.g. isoflurane) and machine
10. Analgesia (e.g. Buprenorphen SR or Meloxicam ER)
11. Female NSG mice 6-8 weeks of age

Tissue implant procedure

From Frozen tissue

1. Thaw tissue in a 37 degree water bath
2. Rinse tissue in DMEM/F12 to remove DMSO from freezing solution
3. Transfer tissue using sterile forceps into a new container

From living tissue

1. Euthanize mice according to protocol (CO2 is sufficient)
2. Sterilize mouse abdomen with 70% EtOH
3. Carefully cut into the skin and peel back the skin to reveal the tumor (Take care to keep the tumor sterile)
4. Remove the tumor to a sterile 10cm-dish on ice
5. Using sterile razor blades mince the tumor into 1-2mmx1-2mm fragments, avoiding regions of tissue with heavy necrosis. Note: (scalpels can work but we find standard razor blades to be superior)
6. Add sterile cold DMEM/F12 to wet the tissue pieces to avoid tissue drying out

Implantation

1. Perform anesthesia and analgesia according to the approved IACUC protocol
2. Remove hair from mouse abdomen by shaving or using a depilatory such as Nair (should ensure any non-autoclavable implements are sterilized to the best and avoid using equipment which cannot be sterilized on both immunocompetent and immunocompromised mice)
3. Place the anesthetized mouse abdomen up and sterilize the implantation site with Iodine and 70% ethanol.
4. Make a small incision between the midline and the 4th nipple revealing the mammary fat pad
5. Make a small incision in the mammary fat pad to create a pocket

6. Place a piece of Matrigel-coated tissue into the pocket of the fat pad
7. Add approx. 10-20 μ l of Matrigel into the pocket
8. Ensure the tissue stays in the pocket, remove tools, and close the site
9. Close the wound with suture or wound clips.
10. Repeat on the opposite side if bilateral implantation is intended
11. Apply a thin layer of triple antibiotic ointment on the incision site
12. Keep mice warm and observe mice for recovery until awakened from anesthesia.
13. Monitor mouse for recovery and additional analgesia per IACUC best practices
14. Monitor for tumor growth and endpoints per approved protocol

Estradiol water treatment – (follow all IACUC guidelines on housing mice with treated water)

1. Dissolve sterile 17 - beta-estradiol in 100% Ethanol to a concentration of 2.4 mg/mL
2. Aliquot into sterile 1.5 mL tubes and store at -20 degrees
3. To supply estrogen at 8 μ g/ml in drink water, add 1 mL of estrogen stock (2.4mg/mL) to 300 ml water in the bottle (final estrogen concentration: 0.8 μ g/ml. Note: NSG mice can be sensitive to high doses of estrogen)