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UABMC - PDX Passage Protocol

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1 Works for me This protocol is published without a DOI.

NCI PDMC consortium

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PROTOCOL CITATION

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GUIDELINES

Purpose

This procedure describes procedure for preparation of fresh primary tumor tissue to establish patient-derived xenograft (PDX) model in mice.

Scope

Fresh tumor samples of tumor tissue will be used to for research in starting and maintaining PDX models serially passaged in mice.

U01 Subgroup:

(Informatics, neurospheres, microtumors, kinomics, RNA-Seq, etc.)

Approvers:

G.Yancey Gillespie, PhD., Christopher Willey. MD, PhD, Anita B. Hjelmeland, PhD

Responsibilities:

Catherine Langford will be responsible for preparation of tissue for injection into mouse flanks to propagate and maintain PDX models in mice from primary tumor tissues.

Sarah Black will be responsible for collection of primary tissues from O.R., and processing of blood and tumor tissue collected for the UAB Brain Tumor Bank, as well as providing samples of tumor to Catherine Langford for further processing for PDX models.

MATERIALS TEXT

Plasticware/Glassware:

Pipettes - Individually wrapped 1ml, 5ml and 10ml pipettes
Pipette tips - boxes of sterile filter pipette tips: 0-200 µl
Pipettors - standard Pipet-Aid pipettors; calibrated adjustable micropipettes for 0-200 µl tips
Filter systems - 0.22 µm pore size syringe filters. (#0975413, Fisher)

Centrifuge Tubes - 50 ml polypropylene centrifuge tubes (#0644318, Fisher)
One 5ml syringe
19 gauge needle
16 gauge needle
23 gauge needle
Sterile 100mm glass Petri dishes

Media and Enzyme Solutions:
Miltenyi Biotec Tissue Dissociation kit, mouse. Aliquot according to kit direction (#130-096-730, Miltenyi Biotec)
RPMI 1640 medium, Serum-free (#MT15040CV, Fisher)

Other Materials:
One GentleMACS Tissue Dissociator, Miltenyi Biotec
One Miltenyi Tissue rotator
CO_2 (5%), humidified incubator set on 37^{O}C
Certified Laminar Flow Biological Safety Cabinet (LFBSC): certification date:_____
"C" Tubes for GentleMACS machine (#130-096-334, Miltenyi Biotec)
Two #3 scalpel handles with #11 blades, sterile (#089165B, Fisher)
Two Semi-curved forceps, 13cm, serrated, sterile (#089135, Fisher)
Three Semi-curved fine iris scissors, 10.5cm, sterile (#1631, Fisher)
Fisherbrand Cell Strainer, 40 mm (#22363547) with Fisherbrand 50ml centrifuge tube
Sterile convertor sheets, (#NC9532037, Fisher)
Tissu-Mend surgical glue or any surgical veterinary glue; gel is preferable to liquid

SAFETY WARNINGS

1. All fresh human tumors are handled under BSL2 conditions. Work is conducted in the LFBSC using personal protective equipment and avoiding the use of sharps where possible.
2. All materials potentially exposed to human material are treated with a 10% bleach solution for a minimum of 10 minutes, double bagged for autoclaving, or incinerated.
3. To ensure a clean preparation, wear a mask and lab coat and gloves. Sanitize gloves with 3% chlorhexidine frequently, especially after touching a possible contaminated item.

TUMOR HARVEST

1

(This must be done aseptically using sterile surgical techniques

Select 1 to 2 mice that have tumors that measure no more than 8-10mm in greatest diameter, are not ulcerated and appear to have grown in a consistent fashion.

2 Euthanize one mouse at a time with CO2 asphyxiation in designated inhalation chamber attached via a two-stage regulator to a CO2 tank. Perform cervical dislocation or bilateral thoracotomy to ensure the mouse is dead.

3 Spray the mouse with 3% chlorhexidine sanitizing solution (over the sink) and transfer mouse to a sterile convertor cloth. Sanitize the skin around the tumor with betadine scrub solution. Wait at least 2 mins before proceeding.

4 Use sterile forceps and one scalpel with a #11 blade to produce an incision in the skin forming a half-circle about 5mm away from the tumor on the ventral (lower) side of the tumor.

5 Use the forceps to lift the edge of the skin and use a new sterile scalpel with #11 blade to carefully dissect under the tumor freeing it from the underlying muscle tissues. Blunt dissection works best, where possible. Use your ring finger of the hand holding the forceps to push the tumor forward during this dissection.

6 After you have dissected the tumor free from the body of the mouse, continue to dissect the tumor from the overlying

skin.

7 Open one of the glass Petri dishes and set the cover aside. Continue the dissection of the tumor such that it "drops" into the open dish. Cover the Dish and transfer to a laminar flow biological safety cabinet.

8 Dice tumor into large pieces (chunks) and dissect out any necrotic (dead) tissue. Necrosis can be white, yellowish or dark red and bloody. Transfer healthy-looking tumor tissue chunks to a new glass Petri dish, if necessary. Place Petri dish on an ice bath until all tumor chunks have been harvested and processed to this point.

9 Place the carcass in the black plastic sack and proceed to euthanize the next mouse and repeat the process. Ensure that the carcass bag is well tied and disposed in the Morgue in Animal Resource Program space.

PDX REIMPLANTATION IN MOUSE FLANK

10 Rinse tissue chunks a couple of times with 5-10 ml each of sterile PBS; aspirate as much rinse solution as possible each time from Petri dish.

11 Mince rinsed PDX tissue chunks finely using 2 #11 scalpel blades in a scissor-like action until the pieces are small enough to fit through the bore of a 10ml pipet.

12 Add **200 µl** PBS to the petri dish with the minced tissue.

13 Using a sterile 1ml slip-tip tuberculin syringe (without needle attached), draw up the tissue

We typically implant 100 – 250ul of tissue per mouse. The hub of the needle will have about 100ul of "dead" space, so draw up enough tissue to compensate for this loss.

14 Fit a 16 gauge 1½ inch needle on the syringe tip and expel any air bubbles and excess liquid. Place the syringe on ice.

15 Anesthetize the mice using ketamine/xylazine by intraperitoneal injection or isoflurane by inhalation. (Refer to Anesthesia SOP#_____)

16 Place the mice on a sterile convertor towel on their side, right flank facing up. Apply Chlorhexidine scrub with a sterile cotton-tipped applicator to the skin on the Right flank, wait a couple of minutes, then wipe with sterile isopropanol wipe to remove excess chlorhexidine. Allow alcohol to dry.

17 Re-sanitize your gloves. Pull the skin of the right flank between your fingers, smoothing it out and lift gently but tightly.

18 Using your middle finger as a guide underneath the skin fold, slowly insert the needle just under the skin. The needle should be visible through the skin. Slowly push the desired amount of tissue chunks into the area on the mouse's side between the front and rear legs. Draw the needle out slowly and pinch the skin together with a pair of semi-curved forceps as the needle retracts. Place a small drop of tissue glue on the needle entry wound.

19 Place the mice back in a clean sterile cage; lay the mice on their left side in the cage). Place the cage half on, half off of a heating pad set to low (**84 °F**) until the mice are awake and able to move around normally.