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Jun 08, 2020

UABMC - PDX Dissection 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

Christopher Willey 2020. UABMC - PDX Dissection Protocol. **protocols.io** https://protocols.io/view/uabmc-pdx-dissection-protocol-bg9yjz7w

CREATED

Jun 08, 2020

LAST MODIFIED

Jun 08, 2020

PROTOCOL INTEGER ID

37912

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.

Subgroup:

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

Approvers:

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

Responsibilities:

1. 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.
2. 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 andEnzyme Solutions:
Recipe for GentlMACS Dissociation Solution:
Miltenyi Biotec Tissue Dissociation kit, mouse. Aliquot according to kit directions
RPMI 1640 medium, Serum-free

Recipe for NeuroBasal Complete Medium:
500 ml NeuroBasal medium, serum-free
One 10ml bottle B27 supplement
One 5 ml bottle N2 supplement,
5ml GlutaMax solution
5ml Amphotericin B, (250ug/ml)
100ul EGF, (final concentration = 10ng/ml). Dissolve EGF in 2ml Sterile PBS, divide into 100ul aliquots and store at -20C
100ul FGF-β, aliquot (final concentration = 10ng/ml). Dissolve FGF-β in 2ml Sterile PBS, divide into 100ul aliquots and store at -20C
1ml Gentamycin, (50mg/ml)

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)
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 **00:02:00** 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.

PREPARATION OF ENZYME SOLUTION

10 Add 35ml of serum-free RPMI 1640 to a 50ml centrifuge tube. Add one aliquot of Enzyme D, Enzyme R and Enzyme A to the tube.

11 Sterile filter the enzyme solution using a 5ml syringe equipped with a 0.22um syringe filter into a "C" tube.

DISSOCIATION OF PDX TUMOR TISSUE

12

This should be performed aseptically in a laminar flow biological safety cabinet – Be sure to use gloves, sanitize your gloves with 3% chlorhexidine spray and observe strict aseptic, no-touch techniques

13 Mince PDX tissue finely using two #11 scalpel blades in a scissor-like action.

14 Using sterile curved forceps, transfer the minced tumor pieces to the "C" tube containing filtered dissociation solution

15 Place the "C" tube upside down on the GentleMACS Tissue Dissociation machine set to "m_imp_tumor 02.02. Press "START" and let the machine run.

16 After **00:40:00** , remove the tissue rotator form the incubator and take “C” tube off.

17 Place “C” tube on GentleMACS machine set to “m_imp_tumor 03.02”. Press “START” and let the machine run.

18 Remove “C” tube from GentlMACS machine and place in centrifuge. Centrifuge **300 x g, Room temperature 00:03:00**

19 Remove “C” tube from centrifuge and place under laminar flow hood. Resuspend the pellet and enzyme solution by pipetting 8–10 times using a 5ml pipet.

20 Place a 40um cell strainer on the top of a 50ml centrifuge tube.

21 Add **10 mL** of serum-free RPMI 1640 to the tissues solution in the “C” tube and pipet gently to mix.

22 Slowly add **6 mL** of the cell suspension to the strainer, allowing it to drip through.

NOTE: This process will take 2 – 3 strainers, as the chunks of non-dissociated tissue will clog the strainer. Monitor the strainer and replace as needed.

23 Centrifuge the strained cell suspension **300 x g, Room temperature 00:07:00** and resuspend the pellet in **10 mL** of serum-free RPMI 1640 at room temperature.

24 Count the cells to determine total number of viable cells using your preferred method.

25 Before any medium is added, disperse the pellet to facilitate resuspension of the cells by flicking the bottom of the tube several times with you forefinger while holding the top of the tube.

26 Resuspend the cells in **10 mL** of NeuroBasal Complete medium and place in a T75 (< 1- million viable cells) or T225 flask (> 10 million viable cells), containing the balance of the appropriate amount of medium for the flask (**5 mL** for T75 and **20 mL** for T225).