Neuroblastoma dissociation technique

Date:_____ **Tumor Name**:_____

Diagnosis:

Clinical History:

Procedure:

Surgeon: Pathologist:

Neuroblastoma Tumor Processing – Cell Dissociation with RBC lysis:

1. Tumor rinsed with $PBS^{-/-}$; use sterile scalpels to chop/mince tumor into small pieces/slurry
2. Transfer to 50ml conical tube (s)→ use multiple tubes as needed for adequate dissociation if tumor > 5 grams or dissociate in 200 ml flask.
3. Fill with $PBS^{-/-}$ to 50ml mark
4. Dissociation
    a. Remove Trypsin from -20 freezer and thaw in 37 degree water bath
    b. Add trypsin to each 50 ml conical tube or flask with PBS and tumor
        i. Tumor <5g = 600uL; Tumor 5-10g = 900uL; Tumor >10g = 1200uL
    c. Place in warm 37 degree water bath x 10 minutes
    d. Check to see if dissociated properly (goal is "slimy" when pipetted near tumor specimen). If too thick add more $PBS^{-/-}$ and incubate again.
        i. Total time in water bath_____
    e. Once complete stop dissociation by adding to the tube with the tumor i. STI (equal amount to trypsin added above)
        1. Tumor <5g = 600uL; Tumor 5-10g = 900uL; Tumor >10g = 1200uL
        ii. DNAse – $1/10^{th}$ volume of STI/Trypsin
            1. Tumor < 5g = 60uL; tumor 5-10g = 90uL; tumor > 10g = 120uL
        iii. 1M Magnesium Chloride – $1/10^{th}$ volume of STI/Trypsin
            1. Tumor < 5g = 60uL; tumor 5-10g = 90uL; tumor > 10g = 120uL
        iv. Add DNAse and MagCl in 30uL increments (each) until "slime" (DNA) resolves; mix well and allow to sit at room temp 2-3 min between each addition.
5. Filter with a 40uM cell strainer (with lip) over the erylenmeyer flask = _____mL cell suspension
6. View under microscope to determine viability
    a. If many clumps, use 20ml syringe and 22 gauge needle to draw up suspension and transfer to another flask
7. Spin cells at 450g (G=RCF) x 5 minutes, aspirate and discard supernatant
8. Add 10ml of RBC lysis solution to each tube with cells.
9. Incubate at room temp x 10 minutes.
10. Mix solution of PBS/10% FBS: add to each tube to a total of 50ml.
11. Spin at 450g x 5 minutes, aspirate and discard supernatant.
12. Resuspend cell pellet in PBS/10%FBS.
13. Count cells – if too many large clumps, use 20guage syringe and 18 guage needle

Neuroblastoma dissociation technique

Date:_____ **Tumor Name**:_____

Cell Count

Count cells and average 4 boxes - _____ x 10,000 = _____x 10^6 cells/mL

_____x 10^6 cells/mL x _____mL = _____ total cells in cell suspension

Preparation of Cells for NB Para-adrenal Injection

1. # of mice to inject: _____
2. Cell concentration: 20,000 cells/uL for NB
3. # of cells needed for injection
    a. Number of mice _____ x 200,000cell/mouse = _____cells
    b. Need extra cells for dead space (60uL)
4. Have $\frac{\text{\_\_\_\_\_ x } 10^6 \text{ cells}}{\text{mL}}$ = Need $\frac{\text{\_\_\_\_\_ x } 10^6 \text{ cells}}{\text{x mL}}$

    x= _____mL of cell suspension for injection + dead space
5. Spin "x mL" cell suspension at 1250 RPM x 10 min
6. Aspirate and discard supernatant
7. Resuspend in matrigel (which was thawed slowly on ice) using a cold pipette tip
    a. Dead space in syringe: 60 uL (to account for possible needle change)
    b. Matrigel needed for injection (NB): _____mice x 10uL = _____uL
    c. Total volume matrigel : 60uL (dead space) + _____(for mice injections) = _____uL
8. Place cells in matrigel on ice
9. Matrigel lot #_____

Ultrasound Guided Para-adrenal Preparation

Take following materials for procedure: syringe (Hamilton 50uL gastight luer lock), 27 1.5 G needles, 22 G catheters, cells suspended in matrigel on ice, cells in media or plain media used to flush syringe

Cryo preserving cells

Resuspend pellet of cells (6 x 10^6 cells/vial for 5 mice) in 10% DMSO/FBS—1ml per vial. Then freeze -80°C in Styrofoam for two days, then transfer into N_2 for long term storage.