

Ewing's Sarcoma dissociation technique

Date: _____ Tumor Name: _____

Diagnosis:

Clinical History:

Procedure:

Surgeon:

Pathologist:

Tumor Processing – Cell Dissociation:

1. Whole tumor (with as little manipulation as possible) placed in tumor press
 - a. Press engaged with contents collected in sterile 6cm plate
 - b. Use warmed DMEM to rinse residual tumor into tumor press
2. Transfer the pressed tissue to a 50-mL conical vial with medium containing warmed DMEM. If tumor size is >5 grams, split into multiple 50-ml conical tubes as needed for adequate dissociation.
 - a. Add 600 microliters of trypsin (if tumor 5-10g add 900uL; tumor > 10g add 1200uL)
 - b. Measure 50 mg of collagenase in eppendorf tube, add 500 microliters of DMEM to eppendorf to dissolve collagenase, then add to 50-ml conical tube containing tumor/DMEM
 - c. Incubate 10min at 37C
 - d. Invert gently: tissue "Falls apart"
 - e. Check to see if dissociated properly (goal is "slimy" when pipetted near tumor specimen). If too thick add more DMEM and incubate again.
 - i. Total time in water bath _____
 - f. 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 – 1/10th volume of STI/Trypsin
 1. Tumor < 5g = 60uL; tumor 5-10g = 90uL; tumor > 10g = 120uL
 - iii. 1M Magnesium Chloride – 1/10th 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.
3. Filter with 40uM strainer
4. Microscope: determine viability
5. Spin cells at 450g (G=RCF) x 5 minutes, aspirate and discard supernatant
6. Add 10ml of RBC lysis solution to each tube with cells.
7. Incubate at room temp x 10 minutes.
8. Mix solution of PBS/10% FBS: add to each tube to a total of 50ml.
9. Spin at 450g x 5 minutes, aspirate and discard supernatant.
10. Resuspend cell pellet in PBS/10%FBS.

