

RB dissociation technique

Date: _____ SJRB- _____

Tumor Inspection and Dissection:

Description:

Total eyes:

Injection Date: _____

Processing:

- a. Flash Frozen Tissue Pieces - _____
- b. Trizol Piece- _____
- c. EM – _____ MAD: _____,
- d. Histo – _____
- e. Cryo Cell Suspension - _____ vials
 - a. 1×10^6 /vial (goal of 100,000 cells/eye)
- f. Xenograft - _____ animals (CB17SCID mice)
 - a. Cells/eye (5uL per eye): _____

Tumor Processing – Cell Dissociation with RBC lysis:

1. Pick up primary human tumor from surgical suite (Sample should be in media) **OR** remove eye from mouse and harvest tumor per SOP.
2. Dissociation
 - a. Remove Trypsin from -20 freezer and thaw in 37 degree water bath
 - b. Add trypsin to each 15 ml conical tube or flask with RPMI and tumor
 - i. Tumor (regardless of size) = add 900uL trypsin
 - c. Place in warm 37 degree water bath x 10 minutes
 - i. Total time in water bath _____
 - d. Once complete stop dissociation by adding to the tube with the tumor
 - i. STI (equal amount to trypsin added above)
 1. Tumor (regardless of size) =add 900uL STI
 - ii. DNase
 1. Tumor (regardless of size) =add 250 uL DNase
 - iii. 1M Magnesium Chloride
 1. Tumor (regardless of size) =add 250 uL MagCl
 - 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 a 40uM cell strainer (with lip).

RBC LYSIS (SKIP THIS STEP IF PRIMARY HUMAN TUMOR)

4. Spin cells at 500 g (G=RCF) x 5 minutes, aspirate and discard supernatant
5. Add 2ml of RBC lysis solution to each tube with cells.
6. Incubate at room temp x 10 minutes.
7. Mix solution of PBS/10% FBS: add to each tube to a total of 10ml.
8. Spin at 500 g x 5 minutes, aspirate and discard supernatant.
9. Resuspend cell pellet in warm RPMI
10. Count cells –

CELL COUNT

Count cells and average 4 boxes - _____ x 10,000 = _____ x 10⁶ cells/mL

_____ x 10⁶ cells/mL x _____ mL = _____ total cells in cell suspension

Preparation of Cells for Intra-Vitreous Injections

1. # Of mice to inject: _____
2. Cell concentration desired: GOAL ~ 100,000 cells/5uL
3. # Of cells needed for injection
 - a. Number of mice _____ x 100,000 cell/eye = _____ cells
4. Have _____ x 10⁶ cells / mL = Need _____ x 10⁶ cells / mL

x= _____ mL of cell suspension for injection

5. Spin "x mL" cell suspension at 1250 RPM x 10 min
6. Aspirate and discard supernatant
7. Resuspend in appropriate amount of warm RPMI, transfer to small eppendorf for injection

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