

## Ex-Vivo Primary Tumor Protocol

### *Primary Tumor Preparation*

Pediatric Medulloblastoma samples are obtained with varying levels of viability and numbers of cells. Samples with fewer than 1 million viable cells or less than 35% pre-sort viability are not used. Upon acquisition, samples are washed in PBS and put in DMEM-F12 with 10% FBS and antibiotic/antimycotic supplements (aliquots labeled P/S/A, Stock in a 500 mL container in TC freezer as 100x).

Samples are minced with scalpels and further triturated through a 10 mL syringe with decreasing needle size (18 gauge to 22 gauge, a 10 mL pipette and 20 gauge needle are options for tough tumors). These supplies are in the cabinet under the dirty hood. Samples are then filtered through a 70  $\mu$ M nylon mesh (above TC centrifuge) and counted prior to NCAM sort.

### *NCAM Sort*

Columns are in a box in the bottom drawer of my bench. Everything else is on the benchtop. Make a Sort buffer: PBS + 0.5% bsa and 2mM EDTA. Resuspend up to  $10^7$  viable cells in 80  $\mu$ L sort buffer. Add 20  $\mu$ L of CD56 MicroBeads. Mix well and incubate in deli case for 15 minutes. In the meantime, add 500  $\mu$ L sort buffer to column to wet it. Wash cells with 15 mL of sort buffer, pellet and resuspend in 500  $\mu$ L sort buffer. Load this onto the column. Wash with 3x 500  $\mu$ L and let it flow through. Remove column from magnetic block, add 1 mL buffer and plunger it out into an eppendorf tube. Add 4 mL of total media, count viability and cell number and proceed to plate preparation.

### *Plate Preparation*

- Sorted cells are pipetted into 96 well assay plates at either  $1.0 \times 10^4$  or  $2.0 \times 10^4$  cells per well. The ideal density,  $2.0 \times 10^4$  cell per well, requires a stock cell solution at  $4.0 \times 10^5$  cells per mL; from this solution 50  $\mu$ L are added to each well using a multichannel pipettor. Set plates aside while preparing drug solutions.
- Drug stock vials (containing 2  $\mu$ L of drug in DMSO) are found in the leftmost 4 boxes of the top shelf of the new freezer. The concentration written on the vials represents the final concentration obtained after dilution. Positive control working aliquots are labeled with a + with a circle around it. If insufficient tubes remain, thaw a tube with just a + on it; these are just a greater volume at the same concentration. Make 2  $\mu$ L aliquots with a + and a circle around it freeze the extra ones for later.
- The drug stock vials should each be diluted with 1 mL of complete DMEM-F12. DMSO control is made using 2  $\mu$ L DMSO in 1 mL complete DMEM-F12.
- Add 50  $\mu$ L of the diluted drug stock to each well. (This 50  $\mu$ L plus the 50  $\mu$ L of cells should yield a total of 100  $\mu$ L).

Each plate is left in a dark incubator ( $37^{\circ}\text{C}$  at 5%  $\text{CO}_2$ ) for 48 hours at which point they are assayed for viability using Cambrex's ViaLight HS Proliferation/Cytotoxicity Kit. Results are reported as percentage of viability of Experimental vs. Control (DMSO). Each well then receives 50  $\mu$ L of drug solution

### *ViaLight HS Proliferation/Cytotoxicity Kit\**

First, bring one bottle of Tris AC Buffer and one bottle of ATP releasing reagent up to RT. Reconstitute one vial of ATP monitoring enzyme with 1 mL of RT Tris AC Buffer (included in kit, lower left deli case in main lab). Mix and equilibrate for 15 minutes. Next make 5, 200  $\mu$ L aliquots. You will need about  $2n+1$  aliquots for  $n$  plates; for each aliquot add 5 mL Tris AC Buffer. Wrap foil around the tube with ATP monitoring reagent to protect it from

light. Freeze unused aliquots. It's a little more than you need, but it'll save the experiment if the machine screws up.

Next, add 100 uL of ATP Releasing agent to each well of your plates and proceed to the machine in the Biggins lab (A2-145; security will let you in if it's locked). Instructions are on the machine. Open Excel and minimize it. Open the ViaLight program. Be sure to prime the P Injection port 6 times. After priming, bring up the measurements window, open the integrate window, find and run the "full" protocol in the Dre folder of Vialight. Injection volume should be 50 uL, don't worry about the other parameters. Click run and sit back. After it's done, save the file and under the file menu click excel evaluation. This will port the data to excel. Enable macros, then under LUMI, import the "Raw Data Spreadsheet". Save the resultant Excel Files and email them to yourself. Repeat for this for each plate and follow the posted instructions to wash the injection ports after use. Discard the plates and remaining ATP monitoring Reagent in the Biggin's BioHazard.