

## **Xenograft Tumor Assay Protocol**

Darren Carpizo, M.D.

- 1) Determine the number of cells for injection (ie  $5 \times 10^6$ ) to determine the number of plates that will require trypsinizing (usually a 100% confluent plate of  $100\text{mm}^2$  will yield at least 2 injections at  $5 \times 10^6$  cells/injection)
- 2) Trypsinize the number of plates to be counted all at once
- 3) Collect detached cells in 50 ml conical and spin for 4min at 800 rpm
- 4) Remove sup and resuspend in 25 ml of SFM for counting
- 5) Remove three 100 ul aliquots into 3 separate eppendorfs and dilute each 100 ul 1:5 by adding 400u. of SFM, mix well
- 6) Remove 50 ul of 1:5 dilutions for counting, count each of three dilutions and average the three numbers
- 7) Determine the conc. of cells in cells/ml by using the following formula:

Average counts  $\times 10,000 \times$  dilution factor (5) = #cells/ml

- 8) Determine the volume required to add to achieve final concentration of cells for injection per volume to be injected (ie  $5 \times 10^6$  cells/ 100 ul injection) by first determining the total number of cells in the 25 ml suspension by multiplying the conc. of cells in #cells/ml  $\times 25 =$  total number of cells. Then use the following formula

Total # cells/x volume =  $5 \times 10^6 / 100\text{ul}$ , solve for x = volume to resuspend pellet of cells to achieve desired final concentration (ie  $5 \times 10^6$  cells/ 100 ul)

- 9) Spin down 50 ml conical for 4 min at 800 rpm
- 10) Discard sup and resuspend the pellet in the previously determined volume from step #8.
- 11) Draw up each injection/ mouse in 1 ml syringes in the tissue culture hood prior to going to the animal facility. Place the separate syringes each containing 100 ul on ice (this step minimized the possibility of the cells settling after being resuspended thus altering the concentration of cells.
- 12) Anesthetize each mouse with isoflurane inhalent just prior to injection. Be careful not to over anesthetize as the mice will succumb to respiratory depression. Just the right amount is when they just begin to stop moving, remove them from the source of anesthetic, let them breath pure air for a few seconds then place their noses just adjacent to the opening of the 50 ml conical duing the injection
- 13) Inject