Lab Week 8

TX-100 Cytoxicity as assessed by Alamar Blue

Cell Split for Experiments in Week 8:
This week you will split your cells on Tuesday. You will begin with a flask that is 80% confluent and you will seed 1-T25 flask so that it is 0.7% confluent after attachment. You will also seed 1-96 well plate. We will leave columns 11 and 12 empty to serve as our "no cell control".

T25: You will follow the procedure for trypsinization and from the resulting 3ml of trypsinized-cell-suspension you will take 45µl and add it to 5ml of fresh media.

96-well: After you take what it is needed for the T25 above, add 15ml of D10 to the cell suspension. Mix the diluted cells by inverting 3-4 times and divide the mixture evenly among the wells by adding 0.2ml or 200µl to each.

By this week you should be comfortable with aseptic subculture technique. Each group will be given 20 minutes in the hood to complete their cell split. Any time over the allotted time will result in a 0.5pt penalty on this week's lab grade.

Experiment: *TX100 Treatment*

The goal of this lab is to both become comfortable working with and interpreting the results of the Alamar Blue viability assay, but also to gain a further understanding of the complexities associated with establishing cytotoxicity.

Materials

Nonsterile

- 96-well plate with confluent HFF cells
- D10 media in a 50ml falcon (must label non sterile after opening at bench)
- 10% Triton X-100 stock solution
- waste container for tips
- media waste container

Procedure

1. Working at the bench, remove the media from wells using a multichannel or single channel pipettor. NOTE: recall that you do not want to leave cells exposed without media for any longer than necessary. So, do this when you are prepared to add your treatment.

2. Prepare your wells according the following concentrations of Triton-X diluted in D10 and totaling 150uL per well: Calculate that volume of D10 needed to reach 150ul. Add the D10, then add the Triton-X. 15mM=10%
    a. Columns 1,2,&12 – 0uL Triton-X
    b. Column 3 – 0.4uL 10% Triton-X (0.025%, 0.0375mM, 2mg/L)
    c. Column 4 – 0.75uL 10% Triton-X (0.05%, 0.075mM, 5mg/L)
    d. Column 5 – 1.5uL 10% Triton-X (0.1%, 0.15mM, 10mg/L)
    e. Column 6 – 3.75uL 10% Triton-X (0.25%, 0.375mM, 25mg/L)
    f. Column 7 – 7.5uL 10% Triton-X (0.5%, 0.75mM, 50mg/L)
    g. Column 8 – 15uL 10% Triton-X (1%, 1.5mM, 100mg/L)
    h. Column 9 – 38uL 10% Triton-X (2.5%, 3.75mM, 250mg/L)
    i. Column 10 – 75uL 10% Triton-X (5%, 7.5mM, 500mg/L)
    j. Column 11– 150uL 10% Triton-X (10%, 15mM, 1000mg/L)

Note: Column 12 does not have cells in it and this will serve as your no cell control.

3. Wait 15 minutes. Remove the media from wells and then add 200ul fresh media to each well and proceed with the Alamar Protocol.

Pointers

- I strongly recommend using the provided 96-well plate template (on Canvas) to diagram, plan and better visualize the experiment.
- We will demonstrate the use of the plate reader to each group and take your readings today. In the future you may have to take your readings yourself.
- We will be generating survival curves (discussed in lecture) with the x-axis being log (%Triton-X). You can present this as % or mM.

Thought Questions

- We are working at the bench because of space issues. What potential errors could result from working in a non-sterile environment? How can we minimize those errors?
- How is this experiment different than the Trypan Blue viability assay? How is it similar?
- Learning how to present your results in figures and tables for the lab reports is just as important as performing the experiment correctly (well.. maybe almost as important). Using our lecture discussions as a guide, think critically about the BEST way to present your data.