A Judge
9/13

Protocol

Microscopic Count of Viable Cells

An important part of assessing cells and their response to environmental pressures (eg. toxins, drugs) is determining their viability. Trypan Blue is a vital stain used to selectively color dead cells blue. The percent viable cells can then be calculated.

Two approaches are listed below. The first is more accurate but may be more time consuming. The second allows quick evaluation of cellular viability in multi-well plates. For this lab, please use the second protocol.

Protocol #1:

Materials

Sterile

- Trypsinized Cell Suspensions for testing

Nonsterile

- Trypan Blue 0.4%
- Hemocytometer
- Micropipettes
- Tally counter (if available)
- Microscope (Only the Inverted, not the EVOS)

Procedure

1. If treating the cells, you must decide if you will treat cells that are attached to the tissue culture plate/flask or trypsinize the cells prior to treatment. For long treatments, attached cells are suggested. For short treatments (less than 15minutes) you may opt to trypsinize the cells first.
2. Prepare a 100µl aliquot of cells for testing in a microfuge tube or cryovial. Cells should be suspended in either media or 1xPBS. *If the cells have been treated in suspension, you must pellet them away from the treatment solution by centrifugation.*
3. The Trypan Blue dye may contain precipitate and must be centrifuged pellet the unwanted material. Centrifuge the 1.5ml stock aliquot at maximum speed for ~20 seconds.
4. Add 100µl of 0.4% Trypan Blue to your cell sample in the microfuge tube (approximately 1:1 dilution). Mix thoroughly and let stand 5 minutes.
5. Withdraw 10uL of cell-dye solution and carefully load the counting chamber of the hemocytometer, taking care not to make bubbles.
6. Perform a cell count using the techniques previously learned (see protocol "hemocytometry").
7. Calculate the number of cells/mL and the % viability. Remember that you have diluted out your cells (approximately 1:1) with Trypan Blue prior to performing the cell count.
8. Properly clean your bench space.

Protocol #2:

Materials

Nonsterile

- Multi-well plate of cells
- 14ml-1x PBS
- 500µl-Trypan Blue 0.4%
- Microscope (Only the Inverted, not the EVOS)

Procedure

1. Examine your cells under the microscope and grossly prior to staining.
2. Working at the bench, remove D10 media or treatment solution from each well using a transfer pipet and place in biohazard.
3. Wash cells with 1ml PBS and remove.
4. Centrifuge the 1.5ml stock aliquot of 0.4% Trypan Blue Dye at maximum speed for ~20 seconds, to pellet unwanted precipitate.
5. Apply 500ul of 1xPBS and 100ul Trypan Blue dye to each well. Mix briefly and gently by rocking the plate.
6. Allow to sit for 5 minutes and then image under microscope.
7. Calculate approximate % viability by counting **at least** 50 cells.