PC12- AT Cell Culture

A. To Passage Cells:

Once the cells become ~80% confluent, they should be passaged, or split. These cells do not grow well if they are passaged too dilutely, and they easily lift and die if they are left to become too dense. These cells are very clumpy and do not lend themselves well to counting. We passage once every 7 days and feed every other day. For electrophysiological experiments, all cells are replated the day before use onto collagen-coated coverslips and used within 24 hours of plating. Cells are also differentiated with NGF and replated (with trituration) onto collagen-coated coverslips the night before recording. *Note: all other protocols in general section

- Heat the complete PC12 cell media
- After all solutions are warmed (~45 min) and the hood area is sterile, remove all of the complete media from the cells and add fresh media
- Lightly "blow-off" the cells using the media drawn up into a pipette and squirted back onto the cells to dislodge them from the surface of the plate. **DO NOT USE TRYPSIN.**
- Remove all of the cells and solution with a pipette to a 15ml conical tube.
- Spin at 1000 rpm (approx 200 x *g*) for 4 min.
- Remove the supernatant carefully from the pelleted cells.
- (If freezing, skip this section and see freezing instructions.) If plating, add appropriate volume of fresh media down the side of the tube, triturate 5-10x to loosen and disperse the pellet.
- If plating throughout the week for electrophysiological recording, plate to T25's and add complete media to 4 mls. In general, when using a new line of cells, we make a number of different cell dilutions to plate at first: 0.1 ml of 5 mls cell suspension, 0.5 ml of 4 mls, 1ml of 4mls, 1.5 mls of 4 mls. This ensures that I don't lose a line due to over/under plating the cells.
- Place flasks into a 5% CO_2 incubator at 37 °C.