

Anita's Protocol For Splitting/Plating NIH-3T3 Cells

- 1) Flask should be close to confluence at the time of splitting. If you will be plating cells on coverslips, begin by coating them with poly-D-lysine:
 - a) Place coverslip in each well of 6-well plate or dish to be used.
 - b) Add 700ul of poly-D-lysine to the top of the coverslip.
 - c) Incubate at RT for ~10min.
 - d) Remove lysine by aspiration.
 - e) Wash plates 5 times with 2-3ml sterile water.
- 2) Remove media from confluent flask by aspiration.
- 3) Add 3ml of Trypsin/EDTA.
- 4) Incubate at 37C for ~3min.
- 5) During incubation, add 15ml of media to a fresh TC-75 flask and 45ml to a sterile 50ml tube.
- 6) Remove cells from incubator and knock several times to dislodge cells.
- 7) Add 7ml media to flask and agitate to ensure all cells are in solution.
- 8) Add 2ml of media/cell suspension to new flask/media and place at 37C
- 9) Add 5ml of cells to 45ml media in 50ml tube; add 2-3ml from this tube to each well/plate (it is not necessary to have the suspension form a "bubble" over the coverslips).
- 10) Incubate plated cells overnight prior to transfection.