ADHERENCE ASSAY (revised 8/23/02)

Preparing Cells

**prepare a 24 well plate with sterile glass coverslips

1) Grow A549 cells to ~80-90% confluency in a T-75
2) Trypsinize cells, spin down and remove media by aspiration
3) Resuspend cells in 5 ml complete growth media
4) Mix 10 µl of cell suspension with with an equal volume of trypan blue
5) Add 10 µl to hemocytometer and count cells in four corners
6) Average four counts to calculate the cell concentration:

2 x average cell count x 10^4 = number of cells/ml

7) Calculate the volume of cells required to give $2x10^5$ cells/well

volume of cells required (ml) = (2×10^5 cells)(#wells)/conc from hemocytometer

8) Adjust volume to give $2x10^5$ cells/ml (ie 1 ml per well)
9) Add 1 ml of A549 cells into each well with coverslip
10) Allow cells to adhere overnight

Binding Assay

1) Remove media and wash A549 cells 2X with HBSS
2) Add 1 ml of complete growth media WITHOUT antibiotics or FBS to each well and incubate at 37 °C, 5% CO_2

3) Grow bacterial culture to OD_{600} ~ 1.0

Pseudomonas: An OD_{600} of 1.0 is equal to $5x10^8$ cfu/ml
spin down cells and resuspend in cell culture media to give a final concentration of 10^7 cfu/ml

4) Remove media from each well
5) Add 1 ml of bacterial suspension to each well
6) Incubate at 37 °C for desired amount of time
6) Fix coverslip and stain