

Adherence Assay by CFU (A549 cells)

(revised 1/30/01)

PROTOCOL

Day 1

- 1) Culture 2×10^5 A549 cells per well in RPMI1640 Complete Media (+FBS) overnight.
- 2) Start overnight bacterial cultures.

Day 2

- 1) Measure OD600 of bacterial cultures and dilute cells back to OD600 = 0.1
- 2) Trypsinize 3 wells to get A549 cell count
 - add 100 μ l Trypsin/EDTA
 - incubate 3-5 min until cells start to lift off
 - add 100 μ l RPMI1640 +FBS to stop
 - mix 10 μ l of cells with 10 μ l Bromphenol blue and count in hemocytometer
- 3) Replace RPMI1640 +FBS in remaining well with -FBS media to serum starve A549 cells
- 4) Grow bacteria to OD600 = 3-3.5
- 5) Wash bacteria 2X in PBS and resuspend in HBSS
- 6) Dilute bacteria in RPMI1640 (-FBS) to 2×10^7 CFU/ml
1.0 OD600 = 2×10^9 CFU/ml therefore, final OD600 = 0.01
- 7) Add 1 ml of RPMI1640 containing bacteria to each well
- 8) Incubate for desired time at 37 °C
- 9) Wash cells 2X with HBSS
- 10) Add 200 μ l 1% triton X-100
- 11) Incubate at room temp for 10 min
- 12) Add 800 μ l ice cold PBS and mix suspension by pipetting
- 13) Transfer cells to a microfuge tube and place on ice
- 14) Dilute samples and plate onto LB plates