Macrophage Invasion/phagocytosis Assay

Mϕ: RAW264.7
Bacteria: Yersinia
Tissue culture media: DMEM + 10% DFCS + glutamine + 1mM Na Pyruvate

Assay:

1. Mϕ culture:
   RAW's seeded 12-24 h before experiment @ 2.5 x 10^5 mf/well OR 1.5 x 10^5 for microscopy
2. Bacterial cultures
   A. grown O/N @ 26°C shaking in 2xYT media

   B. O/N's subcultured 1:50 in 2.5 ml of 2xYT + 20mM NaOxalate + 20mM $MgCl_2$

   per 10ml = 9 ml 2xYT
   0.8 ml NaOxalate (200mM)
   0.2 ml $MgCl_2$ (1M)

   C. shake @ 26^0C for 2 h followed by a shift to 37^0C shaking for 2 h

Invasion

A. add approx. 2.5x10^6 CFU/well of mϕ (read OD_{600} 1.0=2x10^9 CFU/ml)
B. centrifuge bacteria onto monolayer @ 2000 rpm for 5 min @ RT
C. place infected monolayers @ 370C + 5% CO2 for 90 min OR do time course and fix accordingly.
D. Aspirate media and gently add 1 ml of pre-warmed DMEM containing 100 μg/ml gentamicin to each sample.
E. Return to incubator for 90 min to kill extracellular bacteria
F. Aspirate gentamicin containing media and wash wells 1X with 0.5 ml pre-warmed DMEM
G. Aspirate and add 200 μl of 1% triton X-100 to each well and allow mϕ to lyse for 5 min @ RT
H. Add 800 μl of LB media to each well and titer sample wells appropriately to determine output CFU