Protocol Name: Cell Counting

Category: Chemotaxis

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Source(s):

Time Required: 1 day preparation + 1 day Experimentation

Additional Notes:

Materials

1x Glass Haemocytometer

15ml LB Media

1x 50ml Falcon Tube

Sterile Inoculation Loops

Sterile Pipette Tips

Coverslips

5% Chemgene/Disinfectant

70% Ethanol

Procedure

1) In sterile conditions, add 15ml LB media to a 50ml falcon tube
2) Using a sterile inoculation loop, touch the surface of a colony of the target species and inoculate the LB
3) Incubate at appropriate temperature until OD_{600}=0.1-0.15
4) Sterilise the glass haemocytometer by spraying with 5% chemegene. Wipe dry and spray again with 70% ethanol. Ethanol should be left to evaporate
5) Place a glass cover slip over the centre grid of the haemocytometer
6) Pipette 20µL of bacterial solution into the trough of the haemocytometer under the coverslip by dispensing at the end of the coverslip
    a. The liquid should be drawn under the coverslip by capillary action
7) Set up brightfield microscope and focus on the haemocytometer
8) Using the top left section, count the number of cells in each of the 16 squares
    a. Do not count cells on the bottom or right hand lines in order to avoid double counting
9) Take an average of the 16 squares
10) As each small square is $0.1mm^3$, therefore the number of cells must be multiplied by 10,000 to obtain cells/ml