Counting cells

To seed cells in an appropriate cell density for further experiments, the available cell number/concentration has to be determined by counting cells.

Material

- Cell suspension (e.g. cell pellet resuspended in 1 mL of growth medium)
- Eppendorf cup (optional)
- PBS (optional)
- Pipette tips (10 µL, 100 µL, 1000 µL)

Equipment

- Biosafety cabinet (use of laminar flow hood or clean bench is also possible)
- Pipettes
- Brightfield microscope
- Neubauer counting chamber

Procedure

1. Prepare a Neubauer counting chamber: Mount coverslip onto counting chamber. Proper mounting can be confirmed by visibility of Newton's rings.
2. Add approx. 15 µL of cell suspension to counting chamber.
   Note: To make sure that cells are distributed homogeneously within the suspension, you should mix it by gently pipetting up and down a few times before taking out cells for counting.
   Optional: If you expect to have a highly concentrated cell suspension, it can be diluted for counting. E.g. for a 1:10 dilution, add 90 µL PBS and 10 µL cell suspension to an Eppendorf cup and mix thouroughly. Use this diluted solution for counting.
3. Using a brightfield microscope, count cells within the Neubauer counting chamber.
   Note: For statistically best results, count all 4 big 4x4 squares and calculate the mean number of cells in one big square.
4. Calculate the cell concentration (cells/mL) of your original cell suspension:
   c = mean number of cells in one big 4x4 square * 10 000 * dilution factor (= 1 in case of no dilution)

Calculation example

You diluted your cell suspension 1:10 and counted 4 big squares.
Your counted cell numbers are 58, 53, 49, 56.
Consequently, the mean cell number per big square is: (58+53+49+56)/4 = 54.
Therefore: c = 54 * 10 000 * 10 cells/mL = 5 400 000 cells/mL = $5.4 * 10^6$ cells/mL

Further calculations

When counting cells, you usually need to seed a certain cell number. Once you know the cell suspension's concentration, this can be calculated easily as described in the following.

c: counted concentration of cell suspension
x: desired cell number
V: volume of cell suspension containing the desired cell number
V = x/c

Example:

c = $5.4 * 10^6$ cells/mL

x = 250 000 cells = 0.25 * 10^6 cells
V = (0.25 * 10^6 cells)/(5.4 * 10^6 cells/mL) = 0.046 mL = 46 µL

Notes

- Always wear a labcoat and gloves.