

ChtA- Counting and cutting back cells to 0.5×10^6 cells/ml

Count cells and determine if gfp-labeled strains glow

1. Leave the microscope fluorescent light on at least 30 min.
2. Place 7 μ l on hemocytometer with cover slip already in place
3. Count to approx. 100 cells and record how many cells you counted and how many squares you counted those cells in (100 cells in 25 squares = 10^6 cells/ml)
4. Check all strains under fluorescence to check for glowing and record the percentage of cells in the gfp-labeled strains that glow.
5. Clean hemocytometer with ETOH

*Use a new flask every couple of days

Cutting back cells

1. Pour contents of flask into autoclaved test tube quickly using sterile techniques.
2. Pipette calculated volume from tube and place into new flask.
3. Add HL-5 up to 50 ml
4. Put flasks in incubator and leave tubes on bench.

Example calculation for cutting back to 0.5×10^6 cells/ml:

-Counted 107 cells in 7 squares

$$\frac{x}{25} = \frac{107}{7} \quad x = 382 \qquad \frac{100}{10^6} = \frac{382}{x} \quad x = 3.8 \times 10^6$$
$$\frac{25}{6} = 6.6 \qquad 50 - 6.6 = 43.4 \text{ ml HL-5}$$