

Estimating the concentration of Dicty cells in suspension

0) Prepare suspension of Dicty cells (see protocol for “Cell suspension preparation”).

1) Vortex the suspension for 10 seconds.

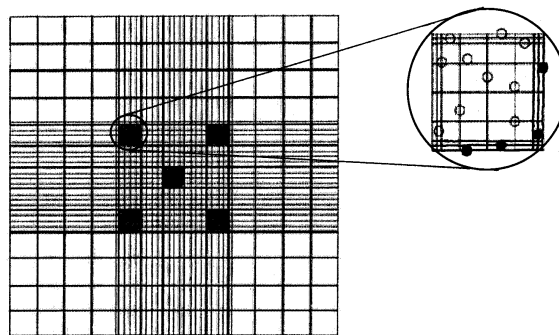
Note: This is important because accurate counts require cells be thoroughly mixed to achieve uniformity. If the suspension is not agitated you may get variable sampling from the original cell suspension.

2) Using cut autoclaved pipette tips deliver a 5 μ l aliquot of the cell suspension to the divot on the hemocytometer. Use a coverslip to pull the droplet over the grid on the hemocytometer.

Note: The volume in the chambers counted is based on the coverslip resting on the sides of the hemocytometer. So be careful not to overflow or inadequately fill the chamber.

3) Place the hemocytometer on the microscope and allow the cells about 30 seconds to settle.

4) Count the total number of cells in each of the five squares indicated below. Count the cells touching the top and left lines. Do not count the cells touching the bottom and right lines (e.g. count all the unshaded cells but not any of the shaded cells in the diagram below). If the initial dilution results in a count of more than 50-100 cells/square, make a further dilution to improve counting accuracy. Generally a 1:10 or a 1:20 dilution is used. For accuracy do not dilute small volumes, for example for a 1:20 dilution dilute a 20 μ l aliquot rather than a 1 μ l aliquot of the cell suspension.



5) Immediately after use, discard the coverslip and rinse the hemocytometer with 70% ethanol and wipe with Kimwipe.

6) Repeat steps 3-6 with another aliquot from the same cell suspension so that you have counts for a total of 10 squares.

7) Repeat steps 2-6 for all other clones.

8) Use following equation to estimate the number of cells/mL in original suspension:

$$\text{Cells/mL (in original)} = (\text{Average no. cells/ square})(25)(10^4)(\text{Dilution Factor})$$