

CELL COUNTING USING A HEMACYTOMETER

Materials:

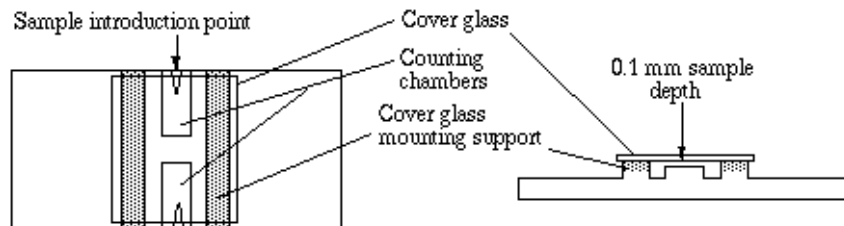
Hemocytometer

cell counter (tally clicker)

cell culture reagents: media, 1xPBS, 1xTrypsin

conical tube and centrifuge

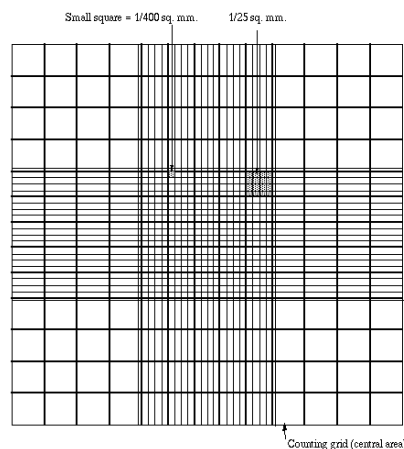
The most widely used type of counting chamber is called a hemacytometer (since it was originally designed for performing blood cell counts).



To prepare the hemacytometer, the mirror-like polished surface is carefully cleaned with lens paper and ethanol. The coverslips (which is thicker than those for conventional microscopy) should also be cleaned. The coverslip is placed over the counting surface prior to adding the cell suspension.

The cell suspension is introduced into one of the V-shaped wells using a pasteur pipet. Allow the area under the coverslip to fill by capillary action. Enough liquid should be introduced so that the mirrored surface is just covered. The counting chamber is then placed on the microscope stage and the counting grid is brought into focus at low power.

One entire grid on standard hemacytometers with Neubauer rulings can be seen at 40x (4x objective). The main divisions separate the grid into 9 large squares. Each square has a surface area of 1mm^2 , and the depth of the chamber is 0.1mm. Each square of the hemacytometer (with cover slip in place) represents a total volume of 0.1mm^3 or 10^{-4}cm^3 . Since $1\text{cm}^3 = 1\text{ml}$, the subsequent cell concentration per ml will be determined using the following calculations.



Cell suspensions should be dilute enough so that the cells do not overlap each other on the grid, and should be uniformly distributed as it is assumed that the total volume in the chamber represents a random sample. This will not be a valid assumption unless the suspension consists of individual well-separated cells. Cell clumps will distribute in the same way as single cells and can distort the result. Unless 90% or more of the cells are free from contact with other cells, the count should be repeated with a new sample. Also, the sample will not be representative if the cells are allowed to settle before a sample is taken. Mix the cell suspension thoroughly before taking a sample to count.

It is best to systematically count the cells in selected squares so that the total count is approximately 100 cells (number of cells needed for a statistically significant count). For large cells this may mean counting the four large corner squares and the middle one. For a dense suspension of small cells you may count the cells in the four 1/25 sq. mm corners plus the middle square in the central square. Additionally, a common convention is to count cells that touch the middle lines (of the triple lines) to the **left** and **top** of the square, but do not count cells similarly on the right or bottom of the square (to avoid double-counting). For cells that overlap a line, count a cell as "in" if it overlaps the top or right line, and "out" if it overlaps the bottom or left line.

To get the final count in cells/ml, first divide the total count by 0.1mm (chamber depth) then divide the result by the total surface area counted (each large square is 1 mm²). For example, if you counted 125 cells total in the four large corner squares plus the middle, divide 125 by 0.1mm, then divide the result by 5 mm².

$$125\text{cells}/0.1\text{mm} = 1250 \text{ cells/mm} \quad (1250 \text{ cells/mm})/ 5\text{mm}^2 = 250,000 \text{ cells/ mm}^3 = 250,000 \text{ cells/ml}$$

Cells per ml = the average count per square x the dilution factor x 10⁴

Sometimes you will need to dilute a cell suspension to get the cell density low enough for counting. In that case you will need to multiply your final count by the dilution factor. For example, if you take an aliquot and dilute it 10-fold and obtain a final count of 250,000 cells/ml, then the count in the original (undiluted) suspension is 10 x 250,000 which is 2,500,000 cells/ml. The cell suspension should be diluted so that each square has between 20 - 50 cells (2-5 x 10⁵ cells/ml). A total of 300 - 400 cells should be counted since the counting error is approximated by the square root of the total count.

To count most mammalian cells on a ~80-90% confluent 100mm TC plate:

1. Rinse cells in 1xPBS.
2. Add 2ml 1xtrypsin and put in incubator ~5min.
3. Add 8 ml of media, pipet up and down to mix cells then transfer to a 15ml conical tube.
4. Centrifuge at 2000rpm for 2 min.
5. Aspirate off media to the desired level:

pellet size	aspirate off to
~50 μ l	2ml
~100 μ l	3ml
~200 μ l	4ml

- Gently resuspend pellet with the remaining media. Mix well to separate clumps, but be gentle with the cells and avoid bubbles.
- Remove a small drop with a pastuer pipet and count cells using the hemacytometer.

For the HeLa HSE-Luc cells, we plated 7000 cells/well on a 96-well plate in 100 μ l.

For a 96-well plate, determine how many cells you will need:

(96wellsx7000cells = approx. 100x7000= 700,000 cells (for a little extra)

$$\frac{\# \text{ cells/ml}}{700,000\text{cells}} = \text{ml of cells needed for one 96-well plate}$$

for a 100 μ l/well on a 96-well plate: ml of cells determined above

30 μ l G418 Geneticin (200 μ g/ μ l)

DMEM/10%FBS to final volume 10ml