Cell Counting

Protocol

Bulletin 6235

Counting Cells with a Hemocytometer and a Microscope

1. Prepare a hemocytometer slide. Place a coverslip in the middle of the hemocytometer.
2. In a micro test tube, combine 10 µl of the cell suspension with 10 µl of TC10™ trypan blue dye. Gently pipet up and down ten times to mix the cells and dye.
3. Load 10 µl of the mixture into the opening of either of the two hemocytometer chambers.
4. Expel a droplet of the suspension and let it be drawn under the coverslip by capillary action.

Note: it is important not to overfill or underfill the chamber.

5. Place the hemocytometer in the microscope and allow the cells about 30 sec to settle.
6. Select the 10× objective and focus on the grid lines in the chamber. Move the slide so that the field you see is on the central area of the grid (central grid area is 1 × 1 mm).
7. Count the cells in the four (4) grid areas labeled as 1, 3, 7, and 9. Are the cells in or out? The usual practice is to include cells overlapping the top and left lines but not those overlapping the bottom or right lines – this eliminates redundant counting if adjacent regions are counted.

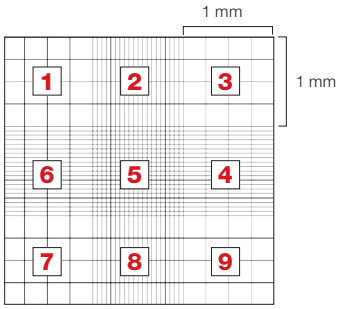

Fig. 1. Neubauer counting grid. The grid is divided in nine 1 mm^2 sections. Numbers indicate the order in which the sections were counted.

12. Count between 100 and 300 cells
13. Calculate the cell concentration in cells/ml; this is equivalent to the average cell count × 2,500

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