Counting cells

A hemocytometer is a specialized microscope slide with a precision-etched grid to determine the number of cells in a known volume. The most common variant for mammalian cells is the Neubauer or “Improved Neubauer” chamber. By staining with a viability dye such as Trypan blue, the proportion of live and dead cells can be determined simultaneously.

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This is a bench card. Full protocol available online.

Procedures

›› *Cell counting with a Neubauer cell chamber*

- ☐ Neubauer cell chamber
- ☐ Hand tally
- ☐ R0133 0.4% Trypan blue solution, 10 µL

(1.) *Optional:* Gently mix the cell suspension with an equal volume of 0.4% Trypan blue.

(2.) Clean the hemocytometer and coverslip with a lint-free tissue to remove dust and debris.

(3.) Moisten the raised glass rails of the hemocytometer. Place the coverslip over the counting surface.

(4.) Carefully load about 10 µL of the sample into the chamber. The void will fill by capillary action.

(5.) Place the hemocytometer on the microscope stage and focus. Cells should be evenly distributed and not overlapping.

- Using the hand tally, count the cells in the four corner grids of the chamber.
- Exclude cells on the left or bottom border, count cells on the right and top border (or vice versa).
- Tally unstained (live) and stained (dead) cells separately.

> ***Quality assurance:*** Repeat with a more concentrated sample if the overall tally is 200 cells or less.

(6.) Average the number of cells across the counted grids and multiply by 1×10^4 mL^{-1} to determine the cell concentration. Be sure to account for any dilution factor introduced by Trypan blue.

Recipe (available online) Resources (available online) Troubleshooting (available online) Notes (available online)