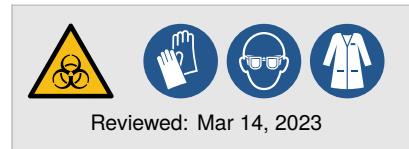


Counting cells

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.



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 $1 \times 10^4 \text{ mL}^{-1}$ 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 \(available online\)](#)

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