

General Protocols: To Count Cells

- ❖ Load the hematocrit with a small volume of the cell suspension (or a diluted volume).
- ❖ With a 10x objective in the phase optics of a microscope, count the cells. There are 9 large 1mm x 1 mm squares. The total cells in one of these 9 squares are counted and multiplied by 10^4 to obtain the number of cells/ml.
- ❖ In practice, count one row of the 5 small squares that comprise the center large square on the 3 x 3 grid of squares. Average the numbers from each of the 5 small squares and multiply by 5 to get the number of cells x 10^4 .

Example: In the 5 smallest squares, the average number of cells counted was 131 cells. $131 \text{ cells} \times 5 = 655 \text{ cells}$, which is $655 \times 10^4 \text{ cells/ml}$ in the cell suspension (which contains 5 mls less the volume to count which is inconsequential). You may want to dilute the initial 5 ml volume by 10-fold to make counting cells a bit easier, however since the cells are fairly clumpy, good resuspension will be key to accurate counting. By viewing the entire 3 x 3 grid of squares, it will be apparent how representative the center square is for all of the squares.