

Counting cells using a hemocytometer

Obtain a viable cell count from suspension cells using a hemocytometer

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Preparing the hemocytometer

1. If using a glass hemocytometer and coverslip, clean with alcohol before use. Moisten the coverslip with water and affix to the hemocytometer. The presence of Newton's refraction rings under the coverslip indicates proper adhesion.
2. If using a disposable hemocytometer (eg INCYTO DHC-N01), simply remove from the packet before use.

Preparing cell suspension

1. Gently swirl the flask to ensure the cells are evenly distributed.
2. Before the cells have a chance to settle, take out 0.5 mL of cell suspension using a 5 mL sterile pipette and place in an Eppendorf tube.
3. Take 100 μ L of cells into a new Eppendorf tube and add 400 μ L 0.4% Trypan Blue (final concentration 0.32%). Mix gently.

Counting

1. Using a pipette, take 100 μ L of trypan blue-treated cell suspension and apply to the hemocytometer. If using a glass hemocytometer, very gently fill both chambers underneath the coverslip, allowing the cell suspension to be drawn out by capillary action. If using a disposable hemocytometer, pipette the cell suspension into the well of the counting chamber, allowing capillary action to draw it inside.
2. Using a microscope, focus on the grid lines of the hemocytometer with a 10X objective.

3. Using a hand tally counter, count the live, unstained cells (live cells do not take up trypan blue) in one set of 16 squares (Figure 1). When counting, employ a system whereby cells are only counted when they are within a square or on the right-hand or bottom boundary line. Following the same guidelines, dead cells stained with trypan blue can be also be counted for a viability estimate if required.
4. Move the hemocytometer to the next set of 16 corner squares and carry on counting until all four sets of 16 corner squares are counted.

Viability

To calculate number of viable cells per mL:

- Take the average cell count from each of the sets of 16 corner squares.
- Multiply by 10,000 (10^4).
- Multiply by five to correct for the 1:5 dilution from the trypan blue addition.

The final value is the number of viable cells/mL in the original cell suspension.

Example

If the cell counts for each of the 16 squares were 50, 40, 45, 52, the average cell count would be

- $(50 + 40 + 45 + 52) \div 4 = 46.75$
- $46.75 \times 10,000 (10^4) = 467,500$
- $467,500 \times 5 = 2,337,500$ live cells per mL in original cell suspension

Figure 1. Hemocytometer gridlines diagram indicating one of the sets of 16 squares that should be used for counting.

