

# Technical Notes—How to Count Fresh Cells

When you receive an AllCells product, please count the cells IMMEDIATELY. AllCells is not liable for any cell loss during subsequent processing. Please note: For Leuko paks, please refer to "How to Count: Leuko pak,," page 192.

## How to Count

In a biosafety hood, use a 5 mL pipet to mix the cell suspension very well and to separate any cell clumps. For any cells in a 1.5 or 2mL tube, mix cells with a P1000 pipet.

Take a 10 µL sample from the vial. Mix the 10µL sample with 10µL of trypan blue. Dilute the cells if necessary and count the number of cells on a hemacytometer to determine the viability.

Table 1: Common Dilution Factors

Tube Size	Cell Number/ Unit Number	Cell Sample	0.4%Trypan Blue Amount	PBS (1x)	Dilution Factor
1.5 to 2mL	0.5 to1 x10 <sup>6</sup>	10µL	10µL	---	2
5mL	5 x10 <sup>6</sup>	10µL	10µL	---	2
15mL	10 x10 <sup>6</sup>	10µL	10µL	---	2
15mL	15 to 20 x10 <sup>6</sup>	10µL	10µL	80µL	10
50 mL	30 to100 x10 <sup>6</sup>	10µL	10µL	80µL	10

N = # of cells counted on all 4 squares of a hemacytometer  
d = dilution factor

## Trypan Blue Method

Equation for Cell Count:

$$\# \text{ of cells/vial} = N / 4 \times 2 \times d \times \text{mL} = \text{mL} \times 10^4$$

Equation for Viability:

$$\# \text{ of cells excluded by trypan blue} / \text{total number of cells} \times 100\% = \text{ \%}$$

Table 2: Common Dilution Factors for MNC Products with RBC (Cord Blood and Bone Marrow Mononuclear Cell Products)

Tube Size	Cell Number/Unit Number	Cell Sample	*3% Acetic Acid with Methylene Blue Solution
50 mL	100-200 x10 <sup>6</sup>	25 µL	475 µL
50 mL	200-400 x10 <sup>6</sup>	25 µL	475 µL

\*StemCell Technologies, Inc., Vancouver; Cat. No. 07060, Tel: 604-877-0713

## Methylene Blue Method

Equation for Cell Count:

$$\# \text{ of cells/vial} \text{ / } 4 \times 20 \times d \times \text{mL} = \text{mL} \times 10^6$$

NOTE: Methylene Blue Method will not give you the cell viability. To find cell viability use Trypan Blue Method.

If your cell count is significantly lower than what AllCells claims, please take the following steps:

1. Check the cell suspension and see if there are any cell clumps. If so, try to separate the cells using a P1000 pipet tip. Most of the cell clumps should be small and easily separable.
2. The optimal cell concentration is 50 to 100 cells on one large hemacytometer square (total 200 to 400 per hemocytometer). Please make the proper dilution or adjust the concentration and count again.
3. Immediately report any significant discrepancies to AllCells.

## Hemacytometer Method

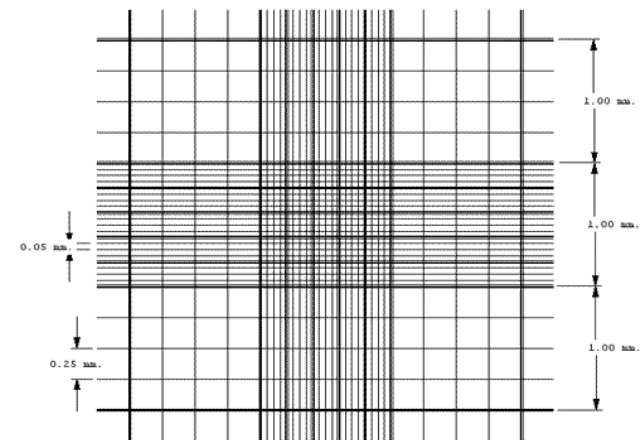
Hausser BrightLine Directions for Use (<http://www.hausserscientific.com/hausserbrightlinedirect.htm>)

Usage: cell counts

Cell Depth: 0.100mm +/- 2% (1/10mm)

Volume: 0.1 Microliter

Ruling Pattern: Improved Neubauer, 1/400 Square mm



Neubauer Ruling (Diagram credit: Hausser Scientific)

Rulings cover 9 square millimeters. The Neubauer ruling boundary lines are the center group of lines (These boundary lines are indicated in the illustration above). The central square millimeter is ruled into 25 groups of 16 smaller squares. Each group is separated by triple lines; the middle group acts as the boundary. The ruled surface is 0.10mm below the cover glass. The volume over each of the 16 small squares is 0.00025 cubic mm.

One (1) Milliliter = 1000 cubic millimeters (cu mm)

One (1) Microliter (µL) = One (1) cubic millimeter (cu mm)

## To clean the counting chamber:

After counting, remove the cover glass and clean the counting chamber with water or a mild cleaning solution (10% solution of bleach). Dry the counting chamber with a soft cloth or wipe, or rinse with acetone.