Protocol

# Estimation of Cell Number by Hemocytometry Counting

Michael R. Green and Joseph Sambrook

The number of mammalian cells in a defined volume of medium can be measured using a hemocytometer. Automated methods using cell-counting devices such as those produced by Coulter are desirable when large numbers of individual samples are to be counted. A hemocytometer contains two chambers, each of which when filled and coverslipped contains a total volume of 9 µL. Each chamber is ruled into nine major squares, and each square is $1 \times 1$ mm with a depth of 0.1 mm. Thus, when coverslipped, the volume of each square is 0.1 $mm^3$ or 0.1 µL. Additional subdivisions of the major nine squares are not necessary for counting and can be ignored.



## MATERIALS

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.**

### Reagents

Cells to be counted
Distilled water
Ethanol (70%)
Growth medium
Trypsin-EDTA (1×)

### Equipment

Coverslip
Hemocytometer
Lens paper
Light microscope with a 10× objective
Pasteur pipettes

## METHOD

*This procedure was adapted from Spector et al. (1998).*

1. Trypsinize the cells by adding two drops (~100 µL) of 1× trypsin-EDTA and incubating for 30–60 sec. Resuspend them in growth medium.

   *See Troubleshooting.*

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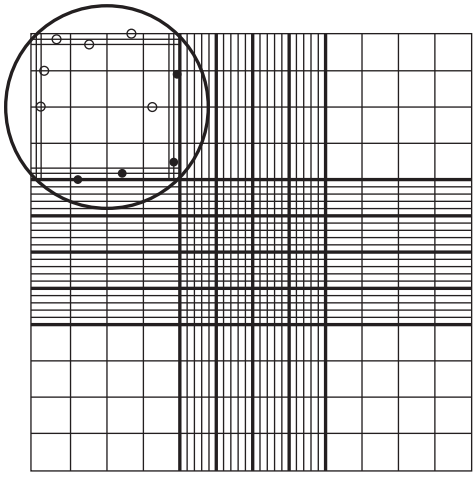

**FIGURE 1.** Standard hemocytometer chamber. The circle indicates the approximate area covered at 100× microscope magnification (10× ocular and 10× objective). Count the cells on *top* and *left* touching the middle line (open circles). Do not count the cells touching the middle line at *bottom* and *right* (closed circles). Count the four corner squares and the middle square in both chambers (only one chamber is represented here). (Adapted from Spector et al. 1998.)

2. Use Pasteur pipettes to remove two independent samples from the cell suspension to be counted. Deliver each sample of cell suspension into one side of the coverslipped hemocytometer by capillary action.

   *Fluid should just fill the chamber and not overflow into the troughs outside the counting face. Load the first sample into one chamber and the second sample into the second chamber.*

   *See Troubleshooting.*

3. Count the total number of cells in five of the nine large squares in each of two sides of the hemocytometer for a total of 10 squares.

   *A representation of the marking on a hemocytometer is shown in Figure 1.*

   *The microscope field using a 10× objective and a 10× ocular should encompass the majority of one of the nine squares of the chamber and is a convenient magnification to use for counting. Cells that overlap the border on two sides of the square should be included in the cell count and not counted on the other two sides. If the initial dilution results in >50–100 cells per square, make a further dilution to improve counting accuracy and speed the process of determining cell numbers.*

   *See Troubleshooting.*

4. Add the number of cells in a total of 10 chambers (five from one side and five from the other) to give the number of cells in $1 \times 10^{-3}$ mL ($1 \times 10^{-4}$ mL per square × 10 squares = a volume of $10^{-3}$ mL). Multiply the total number of cells by 1000 to give the number of cells/mL in the sample counted.

   *If dilutions from the original cell suspension have been made, this factor must also be incorporated (e.g., see Box 1).*

## BOX 1. EXAMPLE

One milliliter of a 10-mL suspension of cells is diluted with 4 mL of medium. The diluted suspension is then sampled with a Pasteur pipette twice. The first sample is delivered to one chamber of the hemocytometer. The second sample is delivered to the second side. Five squares are counted from each side of the hemocytometer.

Number of cells/square : 45, 37, 52, 40, 60, 48, 54, 70, 58, 60

Total count : 524

Dilution factor : $(1 + 4)/1 = 5$

Cells/mL (in original) : $524 \times 10^3 \times 5 = 2.62 \times 10^6$/mL cells

5. Immediately after use, clean the hemocytometer and coverslip by rinsing in distilled $H_2O$ followed by 70% ethanol. Dry with lens paper.

*Do not allow the cell suspension to dry on the hemocytometer.*

## TROUBLESHOOTING

***Problem (Step 1):*** Variable sampling from the original cell suspension.

***Solution:*** The cell suspension must be agitated; do not allow the cells to settle to the bottom of the container.

***Problem (Step 2):*** Inadequate or excessive filling of the hemocytometer chamber.

***Solution:*** The volume in the chambers counted is based on the coverslip resting on the sides of the hemocytometer. Overflow increases the volume counted.

***Problem (Step 3):*** Cell clumping.

***Solution:*** Large clumps of cells may be too large to enter the chamber through capillary action and will be excluded from the cell count. Small clumps that are able to enter the chamber are difficult to count with accuracy. It is important to have a monodisperse suspension of cells for accurate counting. The cells must be thoroughly mixed to achieve uniformity.

## REFERENCES

Spector DL, Goldman RD, Leinwand LA. 1998. *Cells: A laboratory manual, Vol. 1. Culture and biochemical analysis of cells*, pp. 2.8–2.10. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.





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