001

The QIAGEN Guide to Animal Cell Culture

Part V: Counting cells

Welcome to the fifth of a series of articles providing useful hints for culturing animal cells. This article contains considerations for counting cells.

This protocol has been adapted from references 1–3. Protocols in this series are examples of methods for general cell culture and have not been rigorously validated and optimized by QIAGEN. There are many alternative protocols in current use.

Cell counting using a hemocytometer

It is often necessary to count cells, for example, when plating cells for transfection experiments. One method for counting cells is to use a hemocytometer. A hemocytometer contains 2 chambers (Figure 1). Each chamber is ruled into 9 major squares (volume of 0.1 mm^3 or 1 x 10^{-4} ml each). Cell concentration is determined by counting the number of cells within a defined area of known depth (volume).

1. Clean the surface of the hemocytometer with 70% ethanol or another suitable disinfectant, taking care not to scratch the surface of the central area. Dry with lens paper.

2. Clean the coverslip, wet the edges very slightly, lay it over the grooves and central area of the hemocytometer and gently press down.

Tip

It is important that the coverslip is properly attached to obtain the correct chamber depth. The appearance of Newton's rings (bright and dark rings caused by interference in the air between the coverslip and the glass surface of the hemocytometer) will confirm that the coverslip is attached properly.

3. Harvest the cells, either by trypsinization (adherent cell cultures; see *QIAGEN News* 2002 No. 3, 20) or by centrifugation at 200 x *g* for 5 min (suspension cell cultures). Resuspend the cells in an appropriate volume of prewarmed growth medium. At least 10^6 cells/ml are required for accurate counting.

Tip

It may be necessary to centrifuge cells and resuspend in a smaller volume to obtain the desired cell concentration for counting. For adherent cells, it is important to produce a single-cell suspension after trypsinizing. Cell clumping will make counting difficult and inaccurate.

4. Mix the cell suspension sample thoroughly. Using a pipet, immediately transfer 20 µl to the edge of one side of the coverslip to fill one chamber of the hemocytometer. Repeat for the second chamber.

Tip The cell distribution should be homogeneous in both chambers.

Tip The cell suspension is drawn under the coverslip and into the chamber by capillary action. The cell suspension should just fill the chamber. Blot off any surplus fluid without disturbing the sample underneath the coverslip.

5. Transfer the slide to the microscope, and view a large square ruled by 3 lines using a 10x objective and 10x ocular. Count the total number of cells in 5 of the 9 major squares.

Count cells that overlap the top and left border of squares but not those overlapping bottom and right borders (Figure 1). This prevents counting overlapping cells twice. If the cell density is too high, the cell suspension should be diluted, noting the dilution factor.

6. Repeat the counting for the second chamber to give a total of 10 squares.

7. Add the number of cells counted in all 10 squares together to give the number of cells in 1 x 10^{-3} ml. Multiply by 1000 to give the number of cells/ml.

IMPORTANT: If the original cell suspension was diluted for counting, multiply by the dilution factor to obtain the number of cells/ml.

8. Clean the hemocytometer and coverslip by rinsing with 70% ethanol and then with distilled water. Dry with lens paper.

Counting Cells Using a Hemocytometer

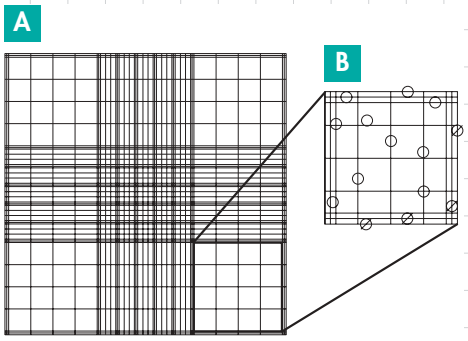

Figure 1 **A** *One chamber of a hemocytometer slide under 10x objective and 10x ocular. The chamber is divided into 9 major squares.* **B** *Detailed view of one of the 9 major squares. Only cells that overlap the top and left borders of squares should be counted to avoid overestimating the cell concentration.* **O***: cells that should be counted;* **Ø***: cells that should be ignored.*

The QIAGEN Guide to Animal Cell Culture will continue in future issues of *QIAGEN News*. If there is any other information you would like to see on these pages of *QIAGEN News*, please let us know by calling QIAGEN Technical Services or your local distributor.

References

1. *Freshney, R.I. (1993)* Culture of Animal Cells: a Manual of Basic Technique. *3rd ed. New York: Wiley-Liss.*

2. *Ausubel, F.M. et al., eds. (1991)* Current Protocols in Molecular Biology. *New York: Wiley Interscience.*

3. *Spector, D., Goldman, R.R., and Leinwand, L.A., eds. (1998)* Cells: a Laboratory Manual. *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.*