Basic cell culture techniques, WB, transient transfection

basic techniques

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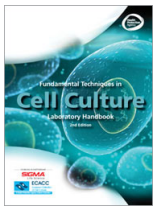
1 Cell Cultures

Watch this video for basic sterile cell culture handling in sterile conditions:

0:00 / 13:21

Further References (optional)

Free online:
From the European Collection of Cell Culture in collaboration with Sigma Aldrich html

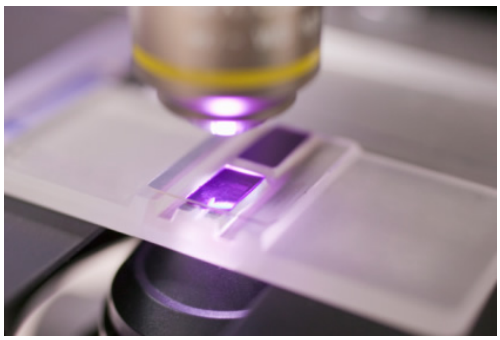


In the library of the Biology department:
Introduzione alle colture cellulari, 2nd edizione, Mariottini *et al.*, Tecniche Nuove, €14,90

previous edition:

2 Cell count

Cell count



Counting Cells with a Hemocytometer

Use of the Hemacytometer for the Determination of Cell Numbers (pdf)

Counting cells by the use of a hemacytometer is a convenient and practical method of determining cell numbers in the case that the Coulter counter is out-of-order temporarily. (It is not that bad.)
The hemacytometer consists of two chambers, each of which is divided into nine 1.0 mm squares. A cover glass is supported 0.1 mm over
these squares so that the total volume over each square is 1.0 mm x 0.1 mm or 0.1 mm^3, or 10^{-4} cm^3. Since 1 cm^3 is approximately
equivalent to 1 ml, the cell concentration per ml will be the average count per square x 10^4.

Hemacytometer counts are subject to the following sources of error:

1. Unequal cell distribution in the sample
2. Improper filling of chambers (too much or too little)
3. Failure to adopt a convention for counting cells in contact with the boundaries lines or with each other (be consistent)
4. Statistical error

- With careful attention to detail, the overall error can be reduced to about 15%. It is assumed that the total volume in the chamber represents a random sample. This will not be a valid assumption unless the suspension consists of individual well-separated cells.
- Cell distribution in the hemacytometer chamber depends on the particle number, not particle mass. Thus, cell clumps will distribute in the same way as single cells and can distort the result. Unless 90% or more of the cells are free from contact with other cells, the count should be repeated with a new sample.
- A sample will not be representative if the cells are allowed to settle before a sample is taken. Always mix the cell suspension thoroughly before sampling.
- The cell suspension should be diluted so that each such square has between 20 - 50 cells (2-5 x 10^5 cells/ml). A total of 300 - 400 cells should be counted, since the counting error is approximated by the square root of the total count.
- A common convention is to count cells that touch the middle lines (of the triple lines) to the left and top of the square, but do not count cells similarly located to the right and bottom.

Hemacytometer counts do not distinguish between living and dead cells.

> A number of stains are useful to make this distinction. Trypan blue among others (Erythrosin B, Nigrosin) can be used: the nuclei of damaged or dead cells take up the stain. If more than 20% of the nuclei are stained, the result is probably significant. Although the trypan stain distinction has been questioned, it is simple and gives a good approximation.

Trypan blu labeling

Materials

- Clean hemacytometer and cover glass, or cover slips
- Balanced Salt Solution (BBS) or PBS
- Trypan blue, 0.4% in BBS (or PBS)
- Microscope
- Tubes
- Hand counter (Colony counter can be used)
- Cell suspension

Procedure

1. Dilute 0.2 ml of Trypan blue with 0.8 ml of BBS.
2. Place cover glass over hemacytometer chamber.
3. Transfer 0.5 ml of agitated cell suspension to a 15 ml tube and add 0.5 ml of diluted trypan blue.
4. With a 1ml pipet, fill both chambers of the hemacytometer (without overflow) by capillary action. Cells will settle in the tube and in the pipet by gravity within a few seconds. Work quickly!
5. Using the microscope with a 10X ocular (and a 10X objective), count the cells in each of 10 squares (1 mm2 each). If over 10% of the cells represent clumps, repeat entire sequence. If fewer than 200 or more than 500 cells are present in the 10 squares, repeat with a more suitable dilution factor.
6. Calculate the number of cells per ml, and the total number of
7. cells, in the original culture as follows:
    - Cells/ml = average count per square x 10^4
    - Total cells = cells per ml X any dilution factor X total volume of cell preparation from which the sample was taken.
    - Repeat count to check reproducibility (+/- 15%).

References

1. Berkson, J., T. B. Magath and M. Hurn (1939). Am. J. Physiol. 128, 309.
2. Sanford. K.K., W.R. Earle, V.J. Evans, H.K. Waltz and J.E. Shannon (1951).
3. Absher, M. in Tissue Culture Methods and Applications, Eds. Kruse, P.F. and
Patterson, M.K., Jr. Academic Press, N.Y., 1973, p.395.

From the Laboratory of Dr. Allan Bradley
Baylor College of Medicine, Houston, Texas

3 Western blotting

Review the basics of WB technique

0:00 / 7:14

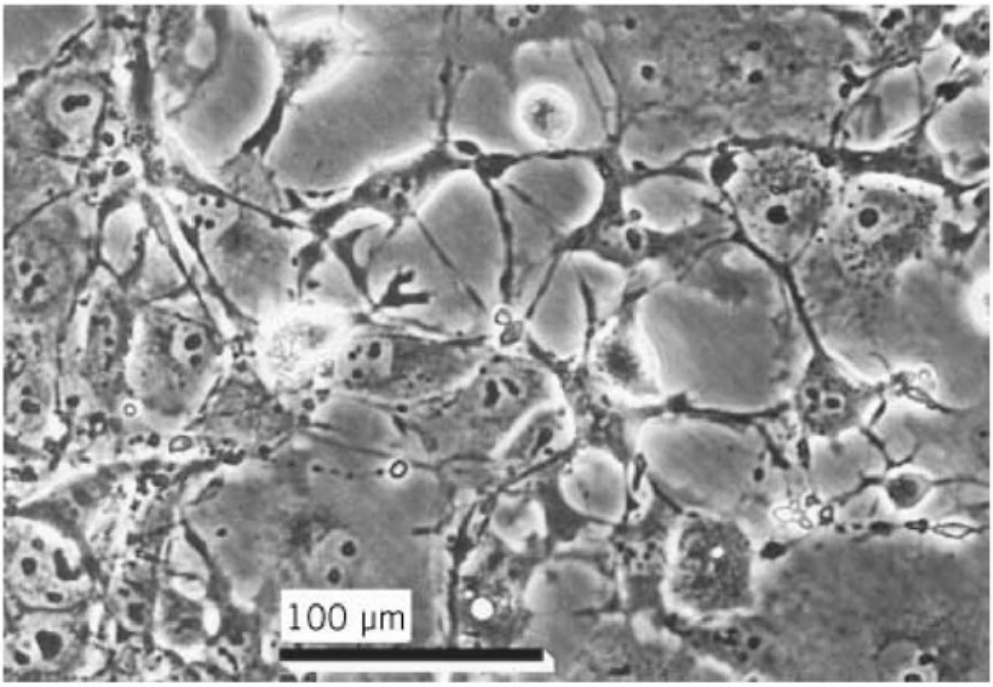
Automatic transcription of the video (txt)

Western blotting in practice:

4 Cos cells

COS cells are monkey kidney fibroblasts (Fig. 1); they have become popular in recent years because they support certain strains of replication-deficient animal viruses and can be transfected easily. Two lines are in common use, COS-7 (ATCC CRL-1651) and COS-1 (ATCC CRL-1650).

Figure 1. Confluent culture of COS-7 cells. Phase contrast, Olympus CK microscope, 20* objective.



1. Origin

COS (an abbreviation for CV-1 in Origin with SV40 genes) cells are a laboratory cell line derived in the 1960's. COS cells behave like fibroblasts, and were originally obtained by immortalizing CV-1 cells from the kidney of the African green monkey (Cercopithecus aethiops) using a SV40 virus that produces large T-antigen but does not replicate correctly.

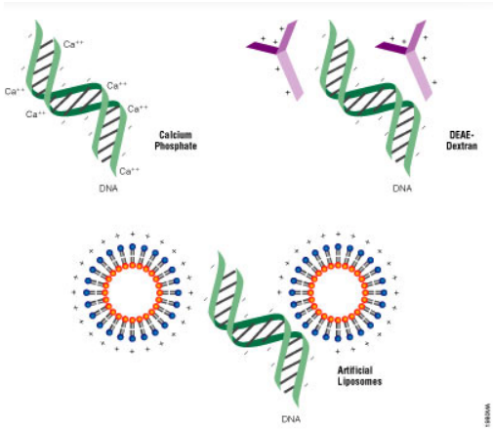
- COS-1 cells contain a single integrated copy of the complete early region of the SV40 genome.
- COS-7 was developed in the 1980's via transformation with a mutant strain of SV40 that codes for the wild-type T-antigen. These cell lines are used to study SV40 monkey viruses and as a common mammalian production cell for recombinant proteins, such as antibodies and signalling molecules.
- COS-1 and COS-7 are T-antigen-positive, permit lytic growth of SV40, and support the replication of temperature-sensitive mutant SV40 tsA209 and of SV40 mutants with deletions in the early region.

2. Properties

COS cells are propagated as a monolayer in the alpha modification of Eagle's minimal essential medium (a-MEM) supplemented with 10% fetal bovine serum (see Serum Dependence) or in Dulbecco's modification of Eagle's medium. They grow rapidly, with an approximate population doubling time of 18h.

3. Usage

COS cells have been used extensively in DNA transfer studies and have been transfected by a number of different methods, including calcium phosphate, DEAE, and lipid transfection.



4. Transient and stable transfection

Transiently transfected cells express the foreign gene but do not integrate it into their genome. Thus the new gene will not be replicated. These cells express the transiently transfected gene for a finite period of time, usually several days, after which the foreign gene is lost through cell division or other factors.

How can you tell whether, or which, cells were transfected successfully? Usually a reporter gene is included in transfection plasmids (or along with other types of nucleic acids, if not using plasmids). Reporter genes indicate the presence of the gene of interest within the cells and can usually be assayed about one to two days after transfection.

Generating stably transfected cells begins with a transient transfection, followed by an infrequent but important and serendipitous process. In a small proportion of transfected cells, the foreign gene is integrated into the cells' genome. The hallmark of stably transfected cells is that the foreign gene becomes part of the genome and is therefore replicated. Descendants of these transfected cells, therefore, will also express the new gene, resulting in a stably transfected cell line.

When developing a stable transfection, researchers use selectable markers to distinguish transient from stable transfections. Co-expressing the marker with the gene of interest enables researchers to identify and select for cells that have the new gene integrated into their genome while also selecting against the transiently transfected cells. For example, a common selection method is to co-transfect the new gene with another gene for antibiotic resistance (such as the neomycin resistance gene, neo) and then treat the transiently transfected cells with the appropriate antibiotic for selection (such as geneticin or G418 for neo-transfected cells). Only the stably transfected cells with resistance to the antibiotic will survive in long-term cultures, allowing for the selection and expansion of the desired cells.