Reducing Serum Levels and Culture Costs

CORNING
Discovering Beyond Imagination

Life Sciences

Introduction

Cell culture is a very important research tool today but one that is also very expensive to use. A major cost component is fetal bovine serum (FBS), an often essential and very expensive part of cell culture. Many researchers routinely use media containing 10 to 20% FBS for growing the cells, but these levels may be higher than cells require. By reducing FBS levels by 50%, researchers may be able to save a considerable amount of money (Table 1). Not all cell lines will do well at lower FBS levels although many will.

This short guide will help you better understand the steps you can take to reduce your cultures' FBS levels and save money while keeping your cultures happy.

Basic Media

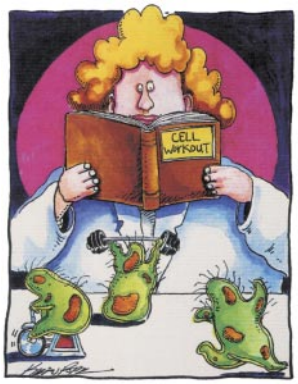
Sometimes high serum levels are necessary because the medium alone does not supply all of the necessary nutrients required by the cells. This is common when a basic "bare bones" medium, such as Eagle's Minimum Essential Medium (EMEM), is used. This widely used medium was developed to determine the *minimum* requirements (as they were known in the 1950s) for highly transformed mouse L-cells to grow in the presence of small amounts of dialyzed serum. It was not designed to promote optimum growth of these cells. Other very basic minimal media include Eagle's Basal Medium (BME) and Dulbecco's Modification of Eagle's Medium (DMEM), which is often confused with EMEM. These media were all designed for

Table 1. Potential Savings from Reducing FBS Concentrations by 50%

Assumptions: Medium costs of $15/500 mL bottle; fetal bovine serum costs $250/500 mL. The serum cost is based on average U.S. list prices for good quality fetal bovine serum from USDA approved countries.

Medium with 10% FBS:		**Medium with 5% FBS:**	
500 mL medium =	$15.00	500 mL medium =	$15.00
55 mL FBS =	$27.50	27 ml FBS =	$13.50
Total =	$42.50	Total =	$28.50

By switching to 5% serum, total media costs are only $28.50/bottle, a total savings of approximately 33% or $14/500 mL bottle.



Good medium is the key to healthy cells!

Figure 1. Cell attachment can be improved without using expensive coatings with the Corning CellBIND Surface.

growing transformed cell lines (HeLa, L-cells etc,) and often do not contain lipids, some vitamins, selenium, zinc, nonessential amino acids, and other trace elements and important micronutrients.

In order to obtain good growth for many widely used cell lines using these basic media, higher levels of serum must be used to supply these missing micronutrients. A prime example of this problem occurs with the widely used CHO cell line. Due to a mutation, these cells have a requirement for proline (a nonessential amino acid) which is not a component of standard EMEM. As a result, to support high levels of growth in these cells, the EMEM needs higher levels of serum from which the cells can obtain proline requirements. Switching to a richer more complex medium with proline will both lower the need for higher levels of serum and save money.

Enriched Media

To help researchers reduce their serum usage, some media manufacturers have taken the traditional basic media formulations and enriched them by adding lipids, insulin, trace metals and other ingredients to develop new proprietary media that are specifically designed to be used with lower serum levels. Although they are sometimes slightly more expensive than basic media formulations, by allowing researchers to reduce serum levels they still save money. Contact your media suppliers for their recommendations.

Attachment Proteins

Besides providing nutrients, growth factors and hormones for the cells, serum also contains fibronectin and vitronectin, two key proteins cells use to interact with a substrate. Thus, when serum levels are reduced, the corresponding reduction in these attachment proteins sometimes leads to a problem with cell attachment. Traditional solutions to this problem have been to either add these attachment proteins to the reduced serum medium or to coat the culture vessels with collagen or other extracellular matrix proteins. However, both of these solutions are very expensive and would eliminate any savings from reducing the serum levels.

Better Surfaces

There is now a third alternative for better cell attachment, the new Corning® CellBIND® Surface. This new surface is created using a patented plasma treatment process to produce a surface that incorporates significantly more (50 to 60%) oxygen than traditional tissue culture surface treatments. Higher oxygen levels increase surface wettability and stability which can lead to better cell attachment, even in very low serum conditions (Figures 1 and 2). By combining the Corning CellBIND Surface with commercially available enriched reduced serum media, researchers can both save money and have "happy" cells.

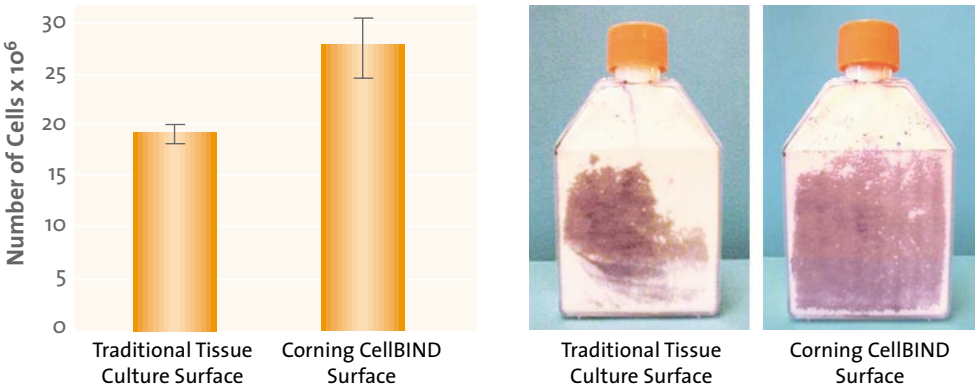
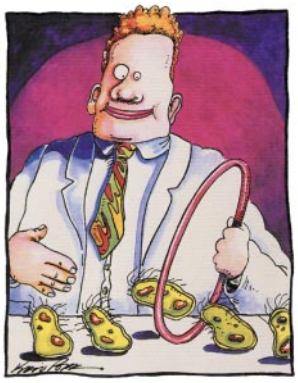


Figure 2. Corning CellBIND Surface Increases HEK-293 cell yields in 1% FBS. Initial seeding density of 1.8 x 10^6 cells/T-75 flask. Cells were grown in 10% serum prior to seeding into IMDM medium containing 1% serum. Cultures grown on the Corning CellBIND Surface had better cell attachment with corresonding 49.5% higher cell yields. Data represents the average count ± SE from six flasks from two separate experiments for each condition tested.

To get more from your cells, give them the best surface!

Figure 3. Larger culture vessels, such as this Corning® CellSTACK® Chamber 10-Stack, should be pregassed to minimize medium pH shifts for faster and more even cell attachment.



Happy cells perform better!

Technique Related Problems

Lastly, serum helps cells survive or recover from harsh treatment by researchers. Poor techniques such as over trypsinization, centrifuging cells too long or hard, leaving harvested cell suspensions at room temperature, all take a toll on cell viability. While using high levels of serum can sometimes reduce or prevent these losses, there is no substitute for good technique and practice.

Some Helpful Hints

If you are currently using high levels of serum (10% or more), you may be able to reduce your serum use by 50% or more while reducing your overall costs by following these simple suggestions.

For better cell attachment and subsequent growth:

1. Try the Corning® CellBIND® Surface to get better cell attachment at lower serum concentrations.
2. Reduce serum levels in several stages, allowing one or two passages at each stage for the cells to adapt, for instance, 10% to 7.5% to 5%.
3. Prewarm medium when initiating cultures to speed up attachment.
4. Pre-equilibrate or pregas culture vessels, especially for larger flasks, roller bottles and Corning CellSTACK® Culture Chambers (Figure 3) to minimize pH increases while cells are initially attaching. The harder it is for cells to initially attach, the more likely there will be uneven attachment and growth.
5. Seed cultures with at least 10,000 to 20,000 cells/cm^2 as a minimum.
6. If cell attachment or slower growth is a problem, try seeding cells at twice their normal density the first few passages until they fully adapt to the reduced serum medium.
7. Harvest cells gently and quickly to avoid damage to the cell surface so that cells can attach faster. Keep exposure to proteolytic enzymes, such as trypsin, as short as possible.
8. Try centrifuging cells more gently at only a 100 xg for only 5 minutes or just long enough to get a soft pellet that is easy to resuspend without damaging the cells.
9. Make sure the dissociating agent has been inactivated or removed by centrifugation. Trypsin is inactivated by proteins in serum but some activity may remain at very low serum levels.
10. Be patient! It may take several passages in the reduced serum medium for the cells to fully adapt.

For happier cells:

1. Grow your cells in a richer, more complex medium. Media manufacturers have developed a variety of enriched media specifically designed to be used at serum levels as low as 3%.
2. Maintain better culture pH levels by using a medium that is supplemented with 5 to 10 mM HEPES organic buffer.
3. Avoid storing medium where it can be exposed to fluorescent lights to prevent formation of hydrogen peroxide and other photoactivated toxic by-products.
4. Buy glutamine-free media when possible and add fresh glutamine solution immediately before use to ensure its stability and freshness. Glutamine has a relatively short half life in medium.
5. Pretest several lots of serum to find the one that is best for your cell lines.
6. Subculture cells before they are confluent, especially epithelial-like cells, so that they have not formed as many tight junctions with other cells and are thus easier to dissociate without lowering their viability.
7. Keep cell suspensions chilled after harvesting, while counting etc. This will increase viability and reduce clumping. Always store frozen cells below -130°C to prevent decreases in culture viability during long term storage.

8. Use enough medium in your culture vessels. We recommend using at least 0.2 to 0.3 mL of medium/cm^2 of growth surface.
9. Keep your cultures well fed. Feed rapidly growing cultures at least twice a week. Better yet, optimize the feeding schedule by measuring glucose depletion (using test strips or meters for monitoring blood glucose) in the medium and feeding when it gets too low. By not overfeeding you can save both time and even more money!
10. Make sure your cultures are not contaminated with mycoplasma. These tiny organisms cannot be seen under the microscope even at concentrations as high as 10^8 mycoplasma/mL but will have a big impact on the health of the cell cultures.

References

1. Mather, J. P. (1998) Making Informed Choices: Medium, Serum, and Serum-Free Medium. *In* Methods in Cell Biology: Animal Cell Culture Methods, Vol. 57, J.P. Mather and D. Barnes, eds. Academic Press, New York, 20-29.
2. Freshney, R. I. (2000) Media. in Culture of Animal Cells: A Manual of Basic Technique. Fourth ed., Alan R. Liss, New York, Chapter 8, 89-104.
3. Waymouth, C. (1974) "Feeding the Baby" – Designing the Culture Milieu to Enhance Cell Stability. J. Nat. Cancer Inst. Vol. 53, No. 5:1443-1448.
4. Ham, R. G. and McKeehan, (1979) W.L. Media and Growth Requirements. *In* Methods in Enzymology: Cell Culture, Vol. 58, W.B. Jacoby and I.H. Pasten, eds. Academic Press, New York, 110-116.
5. For additional information on cell culture surfaces, please visit**www.corning.com/lifesciences/surfaces**.

Cell culture product and technical information is available on the Corning Life Sciences web site **www.corning.com/lifesciences**. Also on our web site, you will find information on the Corning Scientific cell culture seminars that provide novel tips, best practices and proven techniques to help you with your research needs, and you can also request free samples. For additional product or technical information, please e-mail us at CLStechserv@corning.com or call 1.800.492.1110. Outside the United States, call 978.635.2200 or contact your local Corning sales office listed below.

Corning Incorporated
Life Sciences
45 Nagog Park
Acton, MA 01720
t 800.492.1110
t 978.635.2200
f 978.635.2476

www.corning.com/lifesciences

Worldwide Support Offices

ASIA

Australia
t 61 2-9416-0492
f 61 2-9416-0493

China
t 86 21-3222-4666
f 86 21-6288-1575

Hong Kong
t 852-2807-2723
f 852-2807-2152

India
t 91 11 341 3440
f 91 11 341 1520

Japan
t 81 (0) 3-3586 1996/1997
f 81 (0) 3-3586 1291/1292

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2716-0339

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands and All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

Corning Incorporated, One Riverfront Plaza, Corning, New York 14831-0001