Reducing Serum Levels and Culture Costs

CORNING

This guide will help you better understand the steps you can take to reduce your cultures' FBS levels and assist you in cost savings.

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 Modified Eagle's Medium (DMEM), which is often confused with EMEM. These media were all designed for 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 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 allow for cost savings.

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

Assumptions: Medium costs of $20/500 mL bottle; FBS costs $665/500 mL. The serum cost is based on average U.S. list prices for good quality FBS from USDA Safety Tested countries.

Medium with 10% FBS	Cost	Medium with 5% FBS	Cost
500 mL medium	$20.00	500 mL medium	$20.00
55 mL FBS	$73.20	27 mL FBS	$35.20
Total	$93.20	Total	$55.20

By switching to 5% serum, total media costs are only $55.20/bottle, a total savings of approximately 33% or $14/500 mL bottle.

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. 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.

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

Enhanced Surfaces

The Corning® CellBIND® surface enhances cell attachment under challenging conditions, such as reduced-serum or serum-free medium, resulting in higher cell yields. This technology uses a microwave plasma process for treating the culture surface. This process improves cell attachment by incorporating significantly more oxygen into the cell culture surface, rendering it more hydrophilic (wettable) and increasing surface stability.

Technique Related Problems

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.

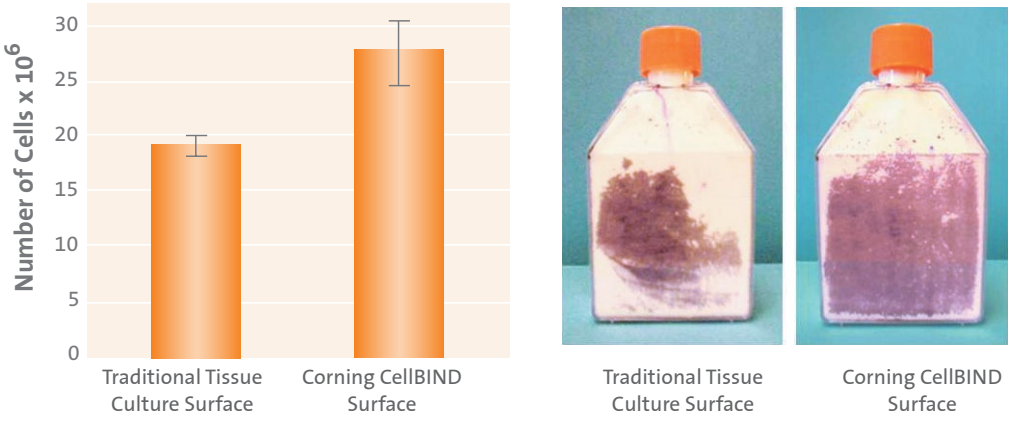

Figure 2. Corning CellBIND surface increases HEK-293 cell yields in 1% FBS. Initial seeding density of 1.8 x 10^6 cells/T-75 flask. Cells were grown in 10% serum prior to seeding into IMDM;medium containing 1% serum. Cultures grown on the Corning CellBIND surface had better cell attachment with corresponding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

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.

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

For a better environment for your 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 JP. (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 RI. (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 RG and McKeehan WL. (1979) 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.

For additional information on cell culture surfaces, visit www.corning.com/lifesciences/surfaces.

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Corning Incorporated
Life Sciences

www.corning.com/lifesciences

NORTH AMERICA
t 800.492.1110
t 978.442.2200

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
Chinese Mainland
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India
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EUROPE
CSEurope@corning.com
France
t 0800 916 882
Germany
t 0800 101 1153
The Netherlands
t 020 655 79 28
United Kingdom
t 0800 376 8660
All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400