Technical Brief:

Adopting Cells to Serum-free Medium

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Introduction

The use of serum-free media has grown significantly in the last 15 years.[1] This is particularly true in industrial applications where the use of serum presents a safety hazard as well as a source of unwanted contamination for the production of biopharmaceuticals.[2] Serum-free medium has several advantages over serum containing medium: simplified and better defined composition, reduced degree of contaminants and elimination of potential sources of infectious agents.[3] Unlike serum-containing medium, serum-free medium is more defined. While composed of many constituents, the composition is known and the level of each component precisely defined. Therefore, the variance seen with serum containing medium is eliminated giving a more controlled environment in which cells can be grown. Drug discovery, physiological, or gene expression studies are simpler to perform with serum-free medium. Serum components are known to bind, degrade or otherwise interact with chemicals added to the medium. Complex associations are possible between the serum, the added effector and the cells. Thus, the effect elicited by the added chemical can be altered or eliminated by the serum factors. Serum-free medium by contrast has fewer possible interferents and where an interaction is observed, more readily controlled. Because the materials used for the production of serum-free media are for the most part highly purified, the risk of contaminating a culture with adventitious agents is greatly reduced or eliminated altogether.

Protocols

There are two approaches for testing the SFMs. The best technique is to use a serum or medium step-down procedure. In this method, the SFM being testing is supplemented with serum or the medium currently being used ("base medium"). At each feeding, the percent of serum or base medium is reduced until the cells are fully adapted to the SFM formulation. Typically, once the serum concentration is below 1.0% the suitability of a given SFM over another will become evident. An alternative method is to subculture the cell line in each of the respective SFM formulations. The formulation giving the best growth is selected as the most suitable and the cells are adapted to that medium using the above procedure. While this is a quick and simple test, not all cell lines will readily adapt to an abrupt shift in medium composition. Therefore, an incremental adaptation to SFM may be more suitable.

Step-Down Protocol

1. Cultivate the cell line to be tested in the base medium employed.
   1.1. If the serum concentration is greater than 5%, reduce the amount of serum to 5%. Once the cells are growing well, proceed to step 2.
2. Transfer the cells to SFM supplemented with 2% FBS (or 1:1 mixture of base medium to SFM).
   2.1. When the cells are growing well, proceed to step #3. If the cells do not grow well, increase the serum concentration to 3 or 4% (2:1 mixture of base medium to SFM) and repeat this step.
3. Transfer the cells to SFM supplemented with 1% FBS (1:4 mixture of base medium to SFM).
   3.1. Once the cells are growing well with 1% serum, continue to step down the serum concentration to 0.75% (1:6 mixture of base medium to SFM) and then to 0.5% (1:10 mixture of base medium to SFM).
   3.2. Adjust the size of the step down increments as needed to maintain good growth. Once the cells are growing well in 0.5% serum (or 1:10 mixture of base medium to SFM), proceed to step 4. It is during this latter series of step down cultivations where difference between the various media will become evident.
   3.3. Choose the media which provides the best growth for the cell line while accounting for product production where relevant.
4. Transfer the cells to SFM without serum or base medium. Passage the cells with a higher inoculum than normal when using SFM. Allow 2-3 feedings before scaling the culture.
   4.1. Notes: For attachment-dependent cells it is strongly recommended that the growth substratum be pre-coated with FNC Coating Mix® or other suitable protein mixture before adding the cells. This becomes more important as the serum concentration is reduced in the medium. It is advisable when performing the step down cultivation, to continue to grow a portion of the cells in the medium from which they came. This is to ensure an adequate supply of adapted cells should the cells not grow in the next step.

Do not use cells which have recovered from a severe lag before growing. This is to avoid selection for good growers which may have picked up a mutant growth phenotype. The use of conditioned medium may help to improve the step down

process as the cells switch metabolism to accommodate the SFM conditions. However, use this sparingly. Mixtures containing 10% to 25% conditioned medium added to the supplemented SFM should suffice.

For cell lines designed for producing a native or recombinant protein, production of the target protein should be monitored during the step down process to ensure that the cell line maintains good expression levels.

Rapid Test Protocol

Caution: Apply this procedure only for cell lines known to tolerate a range of culture formulations.

1. Prepare six cultures of the cell line to be tested.
2. Culture the cells to be tested in the base medium until they reach 50% confluence or 50% maximum cell density.
3. Remove the medium from the cultures and replace it with each of the five media and the base medium.
4. Continue cultivating the cells. Observe the growth over a period of 2-3 days.
5. If the cells can tolerate a particular SFM formulation, continued growth without any aberrant morphologies should be observed.

Product Support

Athena offers a family of serum-free media for the cultivation of mammalian cells. These opened sources formulations allow for the customization of the medium to optimize the growth of specific cell lines. For technical support please contact us at support@athenaes.com.

About the Author

Sheldon E. Broedel, Jr., Ph.D. serves as the Chief Science Officer at AthenaES™. He has over 39 years of research experience, 25 of which were spend in the biotechnology industry. Dr. Broedel has broad expertise in a range of biology disciplines with over 160 products and patents to his credit. Currently, his research teams are developing production tools - reagents, instruments and processes - that increase the production and recovery of recombinant proteins.

Endnotes

1. Broedel, Jr., S. E. and Papciak, S. M. 2003. The case for serum-free medium. BioProcess International. 2:56-58.
2. Merten, O.-W. Safety issue of animal products used in serum-free medium. In Animal Sera, Animal Sera Derivatives and Substitutes Used in the Manufacture of Pharmaceuticals: Viral Safety and Regulatory Aspects. Dev. Biol. Stand. Brown F., Cartwright T., Horaud, F. and Spieser, J.M. eds., Basel, Karger, 1999, Vol. 99:167-180.
3. Froud, S. J. The development, benefits and disadvantages of serum-free media. Ibid. pp. 157-166.

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Serum-free Media Products Information

Catalog Number	Product	Size
0401	BRFF-BMZERO™ - for the cultivation of primary and established cells of epithelial origin	500 mL
0402	BRFF-EPM2™ - for the cultivation of primary and established cells of lung and esophageal origin; suitable for use with airway models	500 mL
0403	BRFF-HPC1™ - for the cultivation of hormone-dependent prostatic cell lines	500 mL
0404	BRFF-P4-8F™ - for the cultivation of neuronal stem cells	500 mL
0410	DMEM / F12 – generic medium formulation supplemented with EGF, insulin, bovine pituitary extract and BSA	500 mL
0411	IMDM - generic medium formulation supplemented with EGF, insulin, bovine pituitary extract and BSA	500 mL
0407	FNC Coating Mix® - is used to coat the growth substratum to increase the attachment and viability of cells	50 mL