DATA SHEET

SFM Screening Kit™

The SFM Screening Kit™

provides a simple way to identify the best serum-free medium formulation for maximum growth of a specific cell line, with five different media and the proprietary FNC Coating Mix® for drastically increasing cell attachment.

SFM Screening Kit™
Application Manual

Table of Contents

Introduction

The use of serum-free media has grown significantly in the last 15 years. 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[1]. 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. 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. Some 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 if an interaction is observed, the interferents can be 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. Athena's SFM Screening Kit™ provides a simple protocol for identifying the best serum-free medium for maximum growth of a specific cell line. It includes five samples of serum-free media, including three proprietary SFM and two general use SFM.

Principle of the Kit

The SFM Screening Kit is intended for researchers seeking to identify the most appropriate serum-free media (SFM) formulation for a specific cell type. While the literature references SFM formulations which have been used to cultivate mammalian cells, unless the same strain as that referenced is employed the best medium may not be known. Further, the SFM formulations used in the past are not necessarily the best formulations for a given cell type. Therefore, it is recommended that several media be tested for suitability before selecting one.

Kit Components

Athena's SFM Screening Kit contains a 100 ml ready-to-use sample of each of five serum-free media, three proprietary media along with two general use formulations. In addition, each kit contains a 25 ml sample of FNC Coating Mix®. Because SFM's are low in protein it is important to pre-coat the growth substratum with proteins as a way of facilitating cell attachment. The FNC Coating Mix® is specially formulated to provide a superior coating than traditional methods by employing a mixture of proteins designed to lay down a matrix which induces cell attachment and proliferation.

SFM Screening Kit™ Components		
Component	**Amount**	**Cat. Number**
BRFF-BMZERO™	100 mL	0401
BRFF-EPM2™	100 mL	0402
BRFF-P4-8F™	100 mL	0404
DMEM / F12	100 mL	0410
IMDM	100 mL	0411
FNC Coating Mix®	25 mL	0407

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 Use Limitations

The SFM Screening Kit™ was designed and is sold for research use only. It should not be used for human diagnosis or drug use or administered to humans unless expressly cleared for that purpose by the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials contained in the kit.

References

1. 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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium