Chrysalis™ Insect Cell Culture: What's the Buzz?

Insect cells are safe, simple and easy to grow…and make fantastic eukaryotic protein factories. Most insect cell-produced proteins have been expressed by employing Baculovirus Expression Vector Systems (BEVS). These are common in both basic research and large-scale commercial applications requiring stable protein expression.

In the late 1940's and 50's, interest in the culture of insect cells was on the rise. Early attempts failed due to the lack of a suitable medium. The right formulation had to be similar to insect hemolymph or "blood." The first successful medium was formulated by G.R. Wyatt for culturing silk moth ovarian tissues. Many formulations followed, but the first true cell line from an insect arose from the pupal tissues of the moth *Antherea eucalypti* by Dr. Thomas Grace in 1962. He modified Wyatt's original formula with supplements and created a new medium known as Grace's insect tissue culture medium, which became widely used due to its success with a broad variety of insect cell lines. Dr. Grace has recently received a lifetime achievement Award from the Society for In Vitro Biology for his work in insect cell culture.

The study of insect cells took off with great success and continued to gain momentum in the following years. More than 500 cell lines from different insect species have been maintained. Insect cell culture has been useful in the studies of morphogenesis, virology, pathology, biochemistry, genetics and many other fields of biology and medicine.

Testing…1, 2, 3

Insect cells are traditionally grown in media that is supplemented with 5-10% serum or other animal-based product. Serum is an excellent source of proteins and other nutrients essential to cell growth. The modern researcher relies on serum for the most sensitive, small batch and newly developed protocols. There is no doubt that serum will deliver the necessary nutrients. When the nutritional value of media under changing conditions is the biggest concern, supplement media with serum to ensure robust cell growth. Gemini's Chrysalis™ insect culture products include an excellent insect cell-tested and qualified FBS (Cat. #100-135) and your choice of Grace's (Cat. #600-310) or TNM-FH Medium (Cat. #600-311).

Pump up the volume

When you're ready to start producing large quantities of proteins, nutrient requirements are more stable and defined, serum-free media should be considered. As a defined product, a serum-free medium minimizes nutritional variables, providing a more controlled culture environment for your cells. The use of serum-free media can eliminate the need for sampling, testing and characterizing lot-to-lot variability, saving both time and money. Serum-free media may also be appropriate for use in the development of therapeutic agents. The introduction of biological variables from animal-derived materials is eliminated in the serum-free approach. Gemini is proud to offer Expression Systems' ESF 921 (Cat. #900-600) and ESF AF (Cat. #900-601) serum-free media.

There are some key points to be aware of when evaluating serum-supplemented vs. serum-free media.

Investment of Time As your cells adapt to a serum-free environment, they will need to be slowly weaned from serum.

Sensitivity Cells grown in serum-free media are more sensitive to changes in temperature, osmolality, pH and shearing.

Antibiotics Overall, antibiotics are ill-advised in serum-free culture. If it is a must, the amount used should be reduced by 5 to 10 fold. In the absence of serum proteins that would normally bind and sequester antibiotic, lower concentrations could prove toxic to cells.

Today, there are many available options in media and sera products for insect cells. If you need an all-purpose medium for a broad range of cell types, use Grace's or TNM-FH. If on the other hand, you intend to produce large amounts of recombinant protein, a serum-free medium might be the preferred way to go.

What does adaptation entail?

There are several different methods for

adapting cells to serum-free culture. The Serum-Halving Method is the most widely used and provides a good conceptual overview. The process takes seven days on average and slowly weans cells away from dependence on animal-derived serum.

1. Culture cells in a medium that is supplemented with 10% FBS until the cells reach the peak of linear log phase.
2. Subculture the cells at the normal ratio into serum-free medium with 5% FBS.
3. When the cells have reached saturation density, subculture in the serum-free medium containing 1% FBS.
4. At each subculture after, reduce the FBS by half, until the concentration is less than 0.06%. At this stage, the cells should be adapted to their serum-free medium.
5. If cell growth declines at any point during adaptation, add serum to the level of concentration that will promote growth.
6. Cell density should never fall below $2x10^5$ or rise above $1.4x10^6$ during the process.