cell culture

New Animal Component-Free, Protein-Free Medium for the Sf21 and Sf9 Insect Cell Lines

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Application Notes

- Animal component-free
- Designed for use with the Sf21 and Sf9 insect cell lines
- Promotes outstanding cell growth
- Supports high level recombinant protein expression with BEVS

Introduction

The utilization of insect cells for the production of recombinant proteins is popular due to the relative ease of use and the ability to make large amounts of protein. Recent advances in creating cell lines with more human-like glycosylation patterns have led to increased interest for biopharmaceutical applications. Cell lines derived from *Spodoptera frugiperda* (Sf) pupal ovarian tissue, such as Sf9 and Sf21, are routinely used in conjunction with the Baculovirus Expression Vector System (BEVS), which takes advantage of viruses that will infect these cell types. The most commonly used baculovirus is *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV). In BEVS, a nonessential baculoviral gene is replaced with the gene of interest and put under the control of a very late viral promoter, such as polyhedrin or p10. During the very late stages of a recombinant baculoviral infection, large amounts of the desired protein can be produced, sometimes reaching 50% of the total insect cell protein.

Traditionally, insect cells are grown in media that contain serum or other animal-derived products. As more recombinant proteins are being employed as therapeutic agents, the methods implemented in their production are coming under increasing regulatory scrutiny. A major area of concern is the presence of animal-derived components in media used to culture cells for recombinant protein expression. Many animal component-free formulations have been developed for other cell culture platforms, such as CHO and NS0; however, the options for insect cell culture are limited. By using an animal component-free medium, the possible threat of adventitious agent contamination from animal-sourced material is eliminated.

Excellent growth and recombinant protein production

In order to meet the demand for an animal component-free, protein-free insect medium, Sigma-Aldrich created TiterHigh™ Sf Insect Medium (Product Code I 5408). This new formulation was designed specifically for the Sf9 and Sf21 cell lines. Utilizing these cell types, TiterHigh Sf Insect Medium was tested against the competition for cell growth and recombinant protein production (β-galactosidase or β-gal) using a recombinant baculovirus. All experiments were performed in duplicate in sterile 125-ml disposable Erlenmeyer shaker flasks (50-ml liquid volume) at 27 °C and 130-rpm shaker speed. Initial cell density was 3 x 10^5 viable cells/ml for the growth assays. The productivity assays were infected at a multiplicity of infection (MOI) of 5 at an initial cell density of 1 x 10^6 viable cells/ml. All cells were adapted to the respective medium prior to experimental set-up. β-gal was quantified using Sigma's β-Galactosidase Reporter Gene Activity Detection Kit (Product Code GAL-A). One-milliliter samples were collected every day and the cells were washed with Hanks' Balanced Salt Solution (HBSS; Product Code H 6648) after centrifugation. The cell lysates were diluted 1:2000.

Figure 1 demonstrates the excellent growth that is attainable in TiterHigh Sf Insect Medium with the Sf21 cell line in shaker culture. Figures 2 and 3 show that TiterHigh Sf Insect Medium also supports excellent recombinant protein production after baculoviral infection. Additionally, TiterHigh Sf will support baculovirus titers of greater than 10^8 PFU/ml.

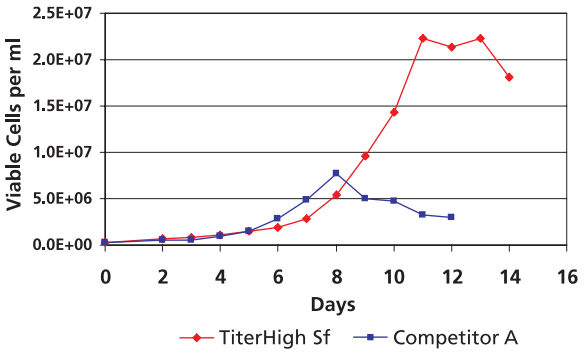

Figure 1. Comparison between TiterHigh Sf Insect Medium and the leading competitor for growth with the Sf21 cell line. *In this experiment Sf21 cells, grown in their respective media, were seeded in Corning® shaker flasks (125 ml) at 3 x 10^5 viable cells/ml. The results show that TiterHigh Sf Insect Medium supports excellent cell growth.*

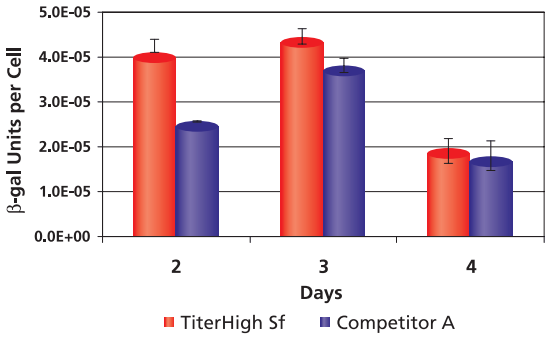

Figure 2. Comparison between TiterHigh Sf Insect Medium and the leading competitor for productivity with the Sf21 cell line. *In this experiment Sf21 cells, grown in their respective media, were infected with a recombinant (β-gal) baculovirus at an MOI of 5 in Corning® shaker flasks (125 ml) at 1 x 10^6 viable cells/ml. The results show that TiterHigh Sf Insect Medium supports high level protein production.*

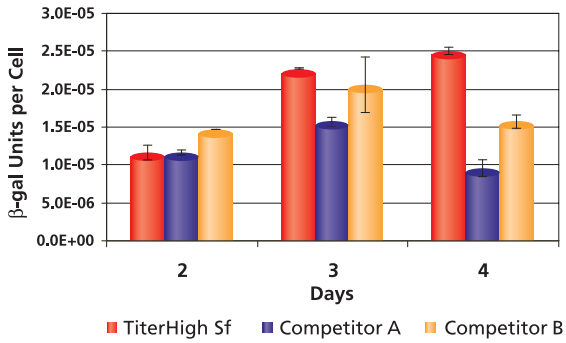

Figure 3. Comparison between TiterHigh Sf Insect Medium and the leading competitors for productivity with the Sf9 cell line. *In this experiment Sf9 cells, grown in their respective media, were infected with a recombinant (β-gal) baculovirus at an MOI of 5 in Corning® shaker flasks (125 ml) at 1 x 10^6 viable cells/ml. The results show that TiterHigh Sf Insect Medium supports high level protein production.*

Summary

TiterHigh™ Sf Insect Medium, Animal Component-free promotes outstanding viable cell densities with Sf9 and Sf21 insect cells and generates high viral titers with both wild-type and recombinant AcMNPV. In addition, this medium supports extremely high levels of recombinant protein production when utilizing BEVS. Finally, as the medium is made without any animal components, potential concerns regarding contamination from adventitious agents are eliminated.

Ordering Information

Product	Description	Unit
I 5408	TiterHigh Sf Insect Medium	1 L
GAL-A	β-Galactosidase Reporter Gene Activity Detection Kit	1 kit
H 6648	Hanks' Balanced Salt Solution	500 ml 1 L