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ProductInformation

TiterHigh™ Sf Insect Medium, Animal Component-Free
Complete Medium, Ready-for-use

Product Code **I 5408**
Storage Temperature 2–8°C

Synonym: TiterHigh™ Sf Insect Medium, AF

Product Description

TiterHigh™ Sf Insect Medium (Product Code I 5408) is an animal component-free, protein-free formulation designed specifically for the *Sf*21 and *Sf*9 (*Spodoptera frugiperda*) insect cell lines. TiterHigh Sf supports fast cell growth rates (doubling times of 20-24 hours), high cell densities of >20 x 10^6 cells/mL for *Sf*21 and >10 x 10^6 cells/mL for *Sf*9, while maintaining high cell viability and high recombinant protein production using the Baculovirus Expression Vector System (BEVS)[1]. TiterHigh Sf Insect Medium will also support high yields of wild-type AcMNPV (occluded and non-occluded forms) and recombinant virus, with and without the presence of 10% Fetal Bovine Serum.

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. With the utilization of TiterHigh™ Sf Insect Medium, regulatory concerns associated with the use of animal-derived components have been eliminated.

TiterHigh™ Sf Insect Medium can be used to grow cells either attached (i.e. flasks and dishes) or in suspension (i.e. shaker flasks, spinner flasks, and bioreactors). It is also capable of supporting long-term cell growth of > 50 passages.

Intended Use

Caution: For manufacturing, processing, or repacking.

Components

The formulation includes inorganic salts, sodium bicarbonate, essential and non-essential amino acids, vitamins, yeast extract, a proprietary lipid formulation, trace elements, and other organic compounds.

It does not contain phenol red, antibiotics, antimycotics, transferrin, and products of animal origin.

Preparation Instructions

This medium is supplied as a complete, sterile-filtered 1X liquid. No supplementation is necessary. The addition of a surfactant (such as Pluronic® F-68) is not required. Glutamine is supplied at 10mM in the form of an Alanine-Glutamine dipeptide. This form is more stable relative to free L-glutamine and is readily accessible to the cells.

Storage/Stability

This medium is stable, when stored 2-8°C and protected from light, until the indicated expiration date on the label. Do not freeze the medium.

Procedure

Freezing and Thawing

Insect cells grown in TiterHigh™ Sf Insect Medium have been successfully frozen in liquid nitrogen and recovered. Cells must be in the mid-logarithmic phase of growth with greater than 90% viability.

1. Pellet cells by centrifugation for 5 minutes at 200 x g. Re-suspend at a concentration of 1 x 10^7 cells/ml in a 1:1 mixture of fresh TiterHigh™ Sf Insect Medium and conditioned TiterHigh™ Sf Insect Medium supplemented with DMSO at a final concentration of 7.5%.
2. Freeze cells via controlled cooling method (1°C decrease per minute) and store frozen cells in liquid nitrogen according to standard procedures.
3. Recover frozen cells by rapidly thawing the vial in a 37°C water bath.
4. Dilute thawed cells 1:10 in fresh TiterHigh™ Sf Insect Medium. Mix and centrifuge suspension at 200 x g for 5 minutes.
5. Re-suspend the pellet in 1 ml TiterHigh™ Sf Insect Medium. Add 9 ml additional fresh TiterHigh™ Sf Insect Medium.
6. Transfer suspension to a T-75 flask containing fresh TiterHigh™ Sf Insect Medium at a final volume of 20 ml. Suspension culture can be transferred to appropriate shaker culture after 2-3 days.

Adaptation to TiterHigh™ Sf Insect Medium

Minimal time is required to adapt insect cells from serum-containing medium to TiterHigh™ Sf Insect Medium. For good adaptation, it is critical that cell viability be >90% and the cells are in mid-logarithmic growth phase. Cells grown in serum-containing medium should be inoculated at a viable cell density of >3 x 10^5 cells/ml in a 1:1 mixture of serum-containing medium and TiterHigh™ Sf Insect Medium. Allow cells to reach a density of 1 to 3 x 10^6 cells/ml. Subculture to an initial density of 3 x 10^5 cells/ml into medium containing increasing proportions of TiterHigh™ Sf Insect Medium, first at a 1:3 ratio and then 1:7 ratio (serum-containing medium: serum-free medium). Cells are considered adapted when the cell density reaches at least 5 x 10^6 cells/ml in serum-free medium and cell doubling rates are <24 hours during log phase of growth. . This usually occurs within 7 days after inoculation. The time interval required for adaptation will vary by individual insect cell lines. The direct adaptation method may also be used by transferring the cells growing in serum directly to TiterHigh™ Sf Insect Medium. However, if sub-optimal performance is observed, the sequential (weaning) method should be used. All cultures should be incubated at 27°C in a humidified atmosphere.

Normally cells can be transferred directly from another serum-free medium to TiterHigh™ Sf Insect Medium, but in some cases adaptation may be necessary. The same procedure outlined for adapting cells from serum can be followed.

Product Profile

Sigma's TiterHigh™ Sf Insect Medium was tested against the competition for cell growth and recombinant protein production (β-galactosidase) using a baculovirus, AcP1-57GAL[3]. All experiments were performed in duplicate in sterile125mL disposable Erlenmeyer shaker flasks (50mL liquid volume) at 27°C and 130rpm shaker speed. Initial cell density was 3 x 10^5 viable cells/mL for the growth assays. The productivity assays were infected at an 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). 1mL samples were collected every day and the cells were washed with 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 *Sf*21 cell line. Figure 2 shows that TiterHigh™ Sf Insect Medium also supports excellent recombinant protein production after baculoviral infection.

Figure 1 Growth Comparison – *Sf*21

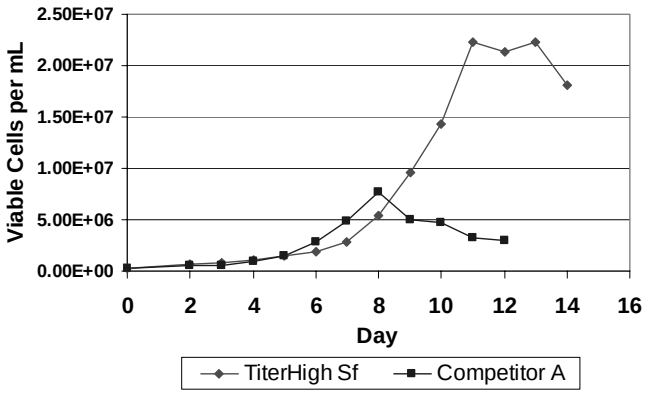
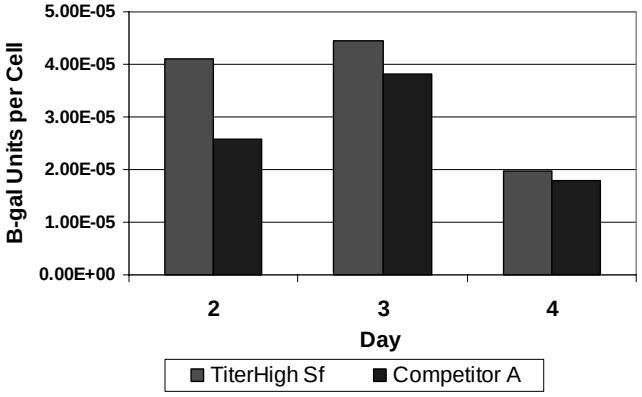


Figure 2 Productivity Comparison – *Sf*21



References

1. Smith, G.E. et al., Production of Human β-interferon in Insect Cells Infected with a Baculovirus Expression Vector. *J. Molecular and Cellular Biology*. **3**, 2156-2165 (1983).
2. Merten, O.W., Safety Issues of Animal Products Used in Serum-free Media. *Dev. Biol. Stand*., **99**,167-180 (1999).
3. Jarvis, D.L. et al., Requirements for Nuclear Localization and Supramolecular Assembly of a Baculovirus Polyhedrin Protein. *Virology*. **185**, 795-810 (1991).

Precautions and Disclaimer

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