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Optimum Growth™ Protocol for Insect Cells

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Introduction to Insect Cell Line Strains

Spodoptera frugiperda Sf9 and Sf21 insect cells are cell lines commonly used for expression of recombinant proteins using baculovirus systems. These cell lines were generated from the parental cell line IPBLSF21, which was derived from the pupal ovarian tissue of the fall army worm (Vaughn, et al. 1977). These cell lines are suitable for transfections, baculovirus expression and amplifications. It is always worth to try protein expression in multiple cell lines, since some cell lines may express more of a particular protein than others (Hink 1991). Tricoplusia ni or High Five insect cells were derived from the cell line BTI-Tn-5B1-4 from ovarian cells of the cabbage looper (GRANADOS, et al. 1994). High Five cell lines are used mainly for protein expression. The S2 cell line was generated from late stage embryos of Drosophila melanogaster (Schneider 1972). This cell line possesses macrophage features. S2 cells grow as a semi-adherent monolayer in tissue culture flasks when compared to the previous cell lines (Sf9, Sf21 and Tn5 which can form adherent monolayers). All of these cell lines can be adapted to suspension cultures. Sf9, Sf21, Hi5, and S2 are all compatible with the Optimum Growth™ flasks (patented).

A great variety of insect cells protein expression systems are available with different vectors that allow different applications (OrbiGen, BD, Clontech, Life Technologies etc).

Start-up culture from Freezer Stocks

Sterile cell culture technique must be used at all times.

In order to start a culture from a frozen stock follow the steps below:

1. Remove frozen vial of cells from liquid nitrogen vapor phase storage and quickly place in 37°C water bath.
2. Remove before fully thawed and spray outside of vial with 70% ethanol before placing it inside a biosafety cabinet.
3. Transfer cells to 125mL Optimum Growth™ flask with 40mL pre-warmed media.
4. Shake flask at 135rpm (1"/25mm orbit) and 27°C .
5. Take a small aliquot of the suspended cells and count them using a ViCell™ to determine density and viability.
6. Continue counting the cells on a ViCell™ every day and passage the cells when they reach the appropriate density depending on the cell line (8eX106 cells/mL for Sf9, 4e X106 cells/mL for Sf21, 3e X106cells/mL for High fives, 3eX107 cells/mL for S2).
7. Passage cells to 1e X106cells/mL once they reach a density of ~8eX106 cells/mL.

Note: The thawed cells may not look nice and round at a first glance and sometimes there might be some cell debris but this will improve with subsequent passages. It is always good practice once the cells have reached at least a third passage to freeze down several cryo-vials of cells as backup.

Freezing Insect Cells

Typically most insect cell lines can be frozen at a density of ~1 x 107 cells/ml.

1. Count the cells using ViCell™ and determine the number of freezer stocks to be made.
2. Centrifuge cells at 600xg for 5 minutes.
3. Remove the media and save.
4. Prepare freezing media and place on ice for > 15 min.
   A. Freezing medium: 45% conditioned (used or spent) medium, 45% fresh medium, 10% DMSO.
5. Resuspend the cells in the cold medium and aliquot 1 ml of cells into 2ml cryo-vials.
6. Put the filled vials in an isopropanol bath (Mr. Frosty) overnight.
7. Placed the freezer stocks in liquid nitrogen.

Passaging or subculturing insect cells

Subculturing is the technique of diluting cells back to a lower density to maintain growth and optimum viability. The state of the cells should be assessed by checking the density and viability before passaging them. Insect cells are typically seeded between 3eX105 cells/mL to 5eX105 cells/mL (high five/Tni) . In order to have consistent growth of healthy cells, they should be in mid-log phase of growth when passaging, especially at when seeding at high densities. This will be well before the cells stop dividing due to high cell density.

Note: Passage cells (SF21, SF9) to 1eX106 cells/mL once they reach a density of ~8eX106cells/mL . After thawing cells you should expand your cell cultures to the maximum volume, without diluting them lower than 1e6 cells/mL, until you reach a volume that is suitable for your needs. Splitting cells into multiple flasks early on is a good idea to minimize the effects of a contamination. Insect cells should be passed every third day to ensure your cells stay in the logarithmic growth phase. To keep your sanity and weekends free you may want to split your cell cultures to 4eX106 cells/mL on Thursdays. This way you only need to pass your cell cultures to 1e X106cells/mL on Mondays and Fridays.

Infecting cell cultures for protein expression by Baculovirus

Baculoviruses are routinely used for recombinant protein expressing in cultured insect

cells. There are a few aspects to consider before starting a protein expression scheme by this system. First, you need to select a

vector that has the promoter suitable for your needs. Then just like it was mentioned earlier, evaluate the insect cells lines, and you can also evaluate different growth media. Lastly, devise a plan that is scalable if large amounts of protein are needed.

Steps to infect insect cell cultures for protein expression:

1. Generate a high-titer virus stock through transfection of bacmid and amplification of the virus (Expression Systems 2011)
2. Determine the titer of your virus (Expression Systems 2011).
3. Perform a small scale- expression test to determine the best multiplicity of infection (MOI) and time of infection (TOI) for expression of the target protein.
4. Passage a 2.5-3.0L of Sf9 cell culture at 1e X106cells/mL in 5L Optimum Growth™ flasks the day prior to infection and shake at 135 RPM and 27°C. *This set up tends to generate the highest yield of monomeric recombinant protein at large scale.

Note: If time or resources do not permit a small scale test of a Baculovirus expression protocol, then typically an MOI of 1-5 and a TOI of 2-3 days works well.

Harvest cell cultures for future protein purification

Once the infection has been done and the culture has reached the end of the TOI:

1. Remove the Optimum Growth™ flasks from the shaker.
2. Take a final cell count of the cultures on the ViCell™.
3. Pour cell cultures into centrifuge tubes and spin down at low speed (~600xg) to prevent cell lysis before downstream processing.
4. For secreted target protein:
5. Decant and save the supernatant.
6. For soluble or membrane target proteins:
7. Decant and save the cell pellet.

Recommended speeds (table 1)

Flask Size	Best Fill Volume	*RPM in 1”/2”
125mL	63mL – 75mL	150/110
250mL	150mL	150/110
500mL	250mL	150/110
1.6L	900mL	150/110
5L	2L – 3L	135/90
1” = 25mm I 2” = 50mm		