*super*Sf9 Insect Cells

An introductory guide to using *super*Sf9 insect cells. Full protocols can be downloaded from our Insect Cell Culture User Guide at www.oetltd.com/product-category/insect-cell-culture/.

Product	Catalogue Number	Size
*super*Sf9-1 Insect Cells	600102	>1 x 10^7 cells/mL
*super*Sf9-2 Insect Cells	600103	>1 x 10^7 cells/mL
*super*Sf9-3 Insect Cells	600104	>1 x 10^7 cells/mL

Product Information

Kit Contents and Preparation

Item	Composition	Storage
*super*Sf9 Insect Cells	*super*Sf9 insect cells (>1 x 10^7 cells/mL) frozen in 50% fresh serum medium (Sf-900™ II), 50% conditioned serum free medium (Sf-900™ II), and Dimethyl Sulfoxide (DMSO) to a final concentration of 7.5%, 2 x 1mL vial	Liquid nitrogen, vapour phase

Product guarantee: 1 year from the date of purchase, when properly stored and handled.

Overview

*super*Sf9 insect cells are derived from the *Spodoptera frugiperda* (S*f*9) cell line and are genetically engineered for the enhanced expression of a wide range of recombinant proteins, including potentially toxic and unstable protein targets. The *super*Sf9 cells come in three varieties; *super*Sf9-1, *super*Sf9-2 and *super*Sf9-3, with each cell line offering unique benefits to promote maximum recombinant protein production. *super*Sf9 insect cells can easily be cultured as monolayer cultures in T-flasks, suspension cultures in shake flasks, or scaled up for use in bioreactors. Like all cells supplied from OET, the *super*Sf9 insect cells are frozen in logarithmic growth with 98% viability. Every batch is measured for growth and viability post recovery from cryopreservation. All master seed banks are tested for sterility and mycoplasma.

Product	Optimised For
*super*Sf9-1 Insect Cells	Offer a prolonged expression time following baculovirus infection. This can help to increase the yield of more stable intracellular, membrane and secreted proteins by up to 15-fold, provided that they are not subject to degredation.
*super*Sf9-2 Insect Cells	Offer a very high expression level over a short period of time following baculovirus infection. This can help to increase the yield of proteins that are more prone to degredation or those that can become toxic to cell health and viability.
*super*Sf9-3 Insect Cells	Offer a high general expression for a variety of recombinant proteins. This can help to increase the yield of recombinant proteins with mixed characteristics but also those with unknown toxicity and stability.

Revival of *super*Sf9 Cells

Updated Oct 2020

The *super*Sf9 insect cells are supplied in 2 x 1mL vial containing >1 x 10^7 cells/mL and transported on dry ice. If the cells cannot be revived immediately upon receipt, the vials should be stored in liquid nitrogen until required. OET supply two vials per order to ensure a back-up is available in the event of any problems when reviving the first vial of cells. If the cells do not appear to be viable, do not attempt to revive the second vial of cells until you have contact OET for advice.

Required by User:

- Insect cell culture growth media warmed to ~28°C (e.g. ESF 921™ [Expression Systems], Sf-900™ II [Gibco®])
- T25 monolayer flasks, vented or non-vented lids
- 125mL cell culture shake flasks, vented or non-vented lids
- Counting chamber or electronic cell counter
- Incubator at 28°C and shaking platform (100-110rpm)
- Water bath at 28-30°C containing fresh/clean water

At OET we recommend initially setting up the cells in an adherent culture and then adapting to suspension culture after two passages. You must use aseptic techniques throughout and work in a class II safety cabinet or tissue culture laminar flow cabinet.

Adherent Culture

1. Defrost the cells rapidly in a 28-30°C water bath until just thawed. Decontaminate the outside of the vial by spraying with 70% ethanol before transferring to a sterile 30mL universal tube containing 10mL of culture medium. Perform a cell count and record the initial cell viability.

2. Divide the 10mL between two T25 flasks to give a final volume of 5mL in each flask. Transfer the flasks to a 28°C static incubator and allow the cells to attach for 45-60 minutes. If using non-vented flasks keep the lids loose to allow for sufficient aeration of the culture.

3. Monitor the cells under the microscope. Live cells will have attached firmly while dead cells will float. Remove the freezing medium and replace with 5ml of fresh growth medium. If cells have not attached after 1 hour, return to incubate overnight.

4. Continue to incubate the cells until they have reached >80% confluency before sub-culturing. Refer to the OET Insect Cell Culture User Guide for advice on the correct confluency for passaging insect cells.

5. Once a confluent monolayer has formed release cells from the flask's surface by tapping the flask sharply against your palm or bench top until >75% of the cells have detached. You can also use gentle pipetting. Do not use a cell scraper to dislodge cells. Transfer 2mL into a new T25 flask containing 3mL of fresh medium (this will create a second adherent culture) and sub-culture the remaining T25 flasks as described in the OET Insect Cell Culture user guide.

Suspension Culture

1. To start a suspension culture, release the cells from two T25 monolayer cultures and transfer the entire volume from one flask, and 3mL from the second flask, to a 125mL shake flask. Count the cells and dilute with fresh growth media to give a final cell count of 1 x 10^6 cells/mL. The total volume in the flask should be between 15-30mL. Use the remaining 2mL in the second T25 flask to continue the cell line as an adherent culture.

2. Incubate both flasks at 28°C, leaving the 125mL flask on an orbital shaker platform rotating at 100-110 rpm. If using non-vented flasks keep the lids loose to allow for sufficient aeration of the culture.

3. Allow the cells to grow for 3-4 days. Count the cells from the suspension flask and transfer to a new 125mL flask the volume of cells necessary to reach a seeding density of 1 x 10^6 cells/mL in 25mL.

4. Once a suspension culture has been established and a cell density of 5 x 10^6 cells/mL has been reached S*f*9 cells are routinely diluted to a density of 0.5-0.8 x 10^6 cell/mL. It is recommended that suspension cultures are passaged 2-3 times and are doubling approximately every 30-45 hours with a viability of >90%, before used for experimental virus work.

Protocols and advice on topics including cell culture techniques, freezing and thawing cells, adapting cells to new media, and establishing new cell cultures can be downloaded from our Insect Cell Culture User Guide at www.oetltd.com/product-category/insect-cell-culture/ *or via our blog oetltd.wordpress.com.*

Product Use

Products are for research purposes only. Not for diagnostic or therapeutic use. For applications including the production of proteins for commercial or diagnostic use including clinical/therapeutic use please contact info@oetltd.com.

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Commercial Purpose include any use of Product in a Commercial Product, the manufacture of a Commercial Product, any resale of the Product, any use (other than evaluation) of Product to facilitate or advance research or development of a Commercial Product, and any use (other than evaluation) of the Product to facilitate or advance any research or development program the results of which will be applied to the development of Commercial Products. “Commercial Product” means any product intended for commercial use.
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