*Sf*9 Insect Cells

An introductory guide to using *Sf*9 insect cells. Full protocols can be downloaded from our Insect Cell Culture User Guide at www.oetltd.com/product-category/insect-cell-culture/.

Product Information

Product	Catalogue Number	Size
*Sf*9 Insect Cells	600100	>1 x 10^7 cells/mL

Kit Contents and Preparation

Item	Composition	Storage
*Sf*9 Insect Cells	*Sf*9 insect cells (>1 x 10^7 cells/mL) frozen in 50% fresh serum-free medium (ESF 921™), 50% conditioned serum free medium (ESF 921™), and Dimethyl Sulfoxide (DMSO) to a final concentration of 10%, 2 x 1mL vial	Liquid nitrogen, vapour phase

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

Overview

*Sf*9 insect cells have become the workhorse of the baculovirus expression system, being used worldwide for the production of recombinant viruses and protein expression. *Sf*9 cells are a clonal isolate from *Spodoptera frugiperda* (Fall armyworm) IPLB-Sf21-AE cells. The *Sf*9 cells are adapted to serum free suspension culture in ESF 921™ media but are capable of conforming to other suitable media types. The cells can be used for transient or stable expression of recombinant proteins; as monolayers for transfection and production of recombinant baculovirus; or for the propagation of baculovirus stocks. *Sf*9 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 *Sf*9 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.

Revival of *Sf*9 Cells

Updated January 2023

The *Sf*9 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

Method:

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 3-6 x 10^6 cells/mL has been reached *Sf9* cells are routinely diluted to a density of 0.5 x 10^6 cell/mL. It is recommended that suspension cultures are passaged 2-3 times and are doubling approximately every 24 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.