OXFORD
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TECHNOLOGIES

Insect Cells

All OET insect cells are ideal for use with both the flashBAC™ expression system and our other range of baculovirus products. Standard Sf9 cells are best suited for large scale virus amplification and protein synthesis, whilst Sf21 cells are recommended for titre based plaque assay and observation of virus cytopathic effects. In addition to this, the Super Sf9 cell line is engineered for enhanced gene expression of more challenging proteins, including those with unstable or toxic characteristics.

Oxford Expression Technologies Ltd
Bioinnovation Hub, Gipsy Lane, Oxford. OX3 0BP
t: +44 (0) 1865 483236 f: +44 (0) 1865 483250
e:info@oetltd.com w: www.oetltd.com

*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.

*Sf*21 Insect Cells

An introductory guide to using *Sf*21 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*21 Insect Cells	600105	>1 x 10^7 cells/mL

Kit Contents and Preparation

Item	Composition	Storage
*Sf*21 Insect Cells	*Sf*21 insect cells (>1 x 10^7 cells/mL) frozen in 50% fresh serum supplemented medium (TC100 with 10% FBS), 50% conditioned serum supplemented medium (TC100 with 10% FBS), and Dimethyl Sulfoxide (DMSO) (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*21 insect cells (IPLB-Sf21-AE) are derived from pupal ovarian tissues of *Spodoptera frugiperda* (Fall armyworm). The *Sf*21 cell line is a suitable host for transient or stable expression of recombinant proteins using baculovirus expression vectors, propagation of baculovirus stocks, and plaque assays to accurately determine virus titre. They are also ideal for monitoring virus cytopathic effects and are more tolerant to sub-optimal growth conditions. *Sf*21 insect cells are adapted to growth in serum-containing medium such as TC100 supplemented with 10% foetal bovine serum (FBS). They can easily be cultured as monolayer cultures in T-flasks or in stirred culture flasks. Like all cells supplied from OET, the *Sf*21 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*21 Cells

Updated January 2023

The *Sf*21 insect cells are supplied in 2 x 1mL vials 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 contacted OET for advice.

Required by User:

- Insect cell culture growth media warmed to ~28°C. At OET we recommend using TC100 media supplemented with 10% FBS
- T25 monolayer flasks, vented or non-vented lids
- Stirred culture flasks (e.g. Techne biological stirrers [www.techne.com])
- Counting chamber or electronic cell counter
- Incubator at 28°C with magnetic stirrer (60rpm)
- 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.

Thawing Cells

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 9mL of culture medium. Perform a cell count and record the initial cell viability.

2. Proceed to set up the cells as either an adherent or stirred/suspension culture.

Adherent Culture

1. 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.

2. 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.

3. 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.

4. Once a confluent monolayer has formed release cells from the flask's surface by tapping the flask sharply against your palm until >75% of the cells have detached. A cell scraper may be used but this can result in decreased viability. Transfer 2mL into a new T25 flask containing 3mL of fresh growth 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.

Stirred Culture

1. This is our preferred method for routine culture of S*f*21 cells. To start a stirred culture, dispense the 10mL of thawed cells into a stirred culture flask and dilute them to a concentration of at least 0.2 x 10^6 cells/mL. The total volume in the flask should be between 25-50mL. A reserve adherent culture may also be set up by transferring 5mL of this stock to a T25 flask as described above.

2. Incubate the flask at 28°C, stirring at 60rpm. It is not necessary to remove the diluted DMSO using this method. 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. After this time the culture should have reached a density of 1-2 x 10^6 cells/mL. We do not recommend exceeding this density before passaging.

4. Once a stirred culture has been established and a cell density of 1-2 x 10^6 cells/mL has been reached S*f*21 cells are routinely diluted to a density of 0.2 x 10^6 cell/mL. It is recommended that stirred cultures are passaged 2-3 times and are doubling approximately every 24 hours with a viability of >90%, before used for experimental virus work. Note that for S*f*21 cells it is acceptable to reuse glass stirred culture flasks following each dilution.

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

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*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 Information

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

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* (*Sf*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 January 2023

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 *Sf*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

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The *super*Sf9 Cell Line ("Product") sold by Oxford Expression Technologies is covered by one or more patents or patent applications owned by the University of Kentucky Lexington which cover components of the Product.
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We grant the purchaser a non-exclusive license to use the enclosed Product for academic research or for commercial evaluation purposes only. The Product is being transferred to you in furtherance of, and reliance on, such license. You may not use the Product, or the materials contained therein, for any Commercial Purpose without a license for such purpose from ParaTechs Corporation who has exclusive licensing rights to the intellectual property and patents associated with the cell line. Inquiries for commercial use should be directed to agoodin@paratechs.com.
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.
Access to the Product must be limited solely to those officers, employees and students of your entity who need access to perform the aforementioned research or evaluation. Each such officer, employee and student must be informed of these terms and conditions and agree in writing, to be bound by same. You may not distribute the Product to others. You may not transfer modified, altered, or original material from the Product to a third party without written notification to and written approval from Oxford Expression Technologies. US patent 7,842,493