Sf9 Insect Cells

Sf9 Insect Cells	3 vials	71104-3

Description

Novagen® Sf9 Insect Cells are derived from *Spodoptera frugiperda* cell line IPLB Sf21-AE. This line has been tested for optimal growth and use in transfections, plaque assays, virus production, and protein expression (1). The cells are frozen using a protocol that optimizes recovery of viable cells after thawing. They are adapted for use in serum-free medium (e.g., BacVector® Insect Cell Medium, Cat. No. 70590), as well as in medium containing serum.

After thawing, Sf9 Insect Cells can be grown as semi-adherent, monolayer cultures in tissue culture flasks, or in suspension as shaker cultures. Cells can be freely transferred between the two culturing methods. Suspension cultures routinely reach densities 2–4 × 10^6 cells/ml with over 95% viability. To maintain experimental consistency, do not passage Sf9 cells indefinitely in culture. It is highly recommended to thaw and culture fresh Sf9 Insect Cells from frozen stock after the current cell culture has exceeded 20–25 passages.

Sf9 Insect Cells and BacVector Insect Cell Medium are recommended for co-transfection of pBAC™ or pTriEx™ plasmids and BacVector Triple Cut Virus DNA to produce recombinant baculoviruses, and for conventional plaque assays using agarose overlays (Table 1). Sf9 Insect Cells may also be used for virus and protein production; however, the combination of TriEx™ Sf9 Cells (Cat. No. 71023) and TriEx Insect Cell Medium (Cat. No. 71022) results in more optimal protein expression and high titer virus stocks with pBAC and pTriEx derived baculoviruses. For more information on those applications, see User Protocol TB314.

Table 1

Application	Sf9 Insect Cells and BacVector Insect Cell Medium	TriEx Sf9 Cells and TriEx Insect Cell Medium
Transfection (plasmid only)	++	+
Co-transfection (BacVector® DNA, pBAC™plasmid DNA and Insect GeneJuice® Transfection Reagent)	+	–
Protein production	+	++
Preparation of high titer virus stocks	+	++
Plaque Assay, conventional	+	–
Plaque Assay, FastPlax™ Titer Kit	+	+

Key: (++) highly recommended, (+) recommended, (–) not recommended

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Europe

France	Germany	Ireland	United Kingdom	All other European Countries
Freephone 0800 126 461	Freecall 0800 100 3496	Toll Free 1800 409 445	Freephone 0800 622 935	+44 115 943 0840

techservice@merckbio.eu

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

www.novagen.com

Components

Sf9 Insect Cells

- 3 vials Sf9 Insect Cells (1×10^7 cells/ml)

Storage of Cells Upon Receipt

Immediately upon receiving the shipment, remove Sf9 Insect Cells from foil pack. Vials may be stored immediately in liquid N_2 for long-term storage (up to 6 months). Transfer cells to a liquid N_2 tank as rapidly as possible.

Alternatively, cells may be placed at –70°C if they will be cultured within 2 weeks (see *Thawing Sf9 Insect Cells* below).

Note: *To prepare exponentially-growing Sf9 cells for long-term storage, see p 3.*

Thawing Sf9 Insect Cells

Medium is defined as serum-free BacVector® Insect Cell Medium. Medium containing serum may be used, if desired. Different lots of medium can vary in their performance. Antibiotics may be used, except where noted in transfection procedures. Some lots of antibiotics may severely inhibit cell growth. We recommend testing new lots for compatibility with cells prior to routine use.

In order to prevent contamination, use sterile techniques when working with uninfected Sf9 Insect Cells and medium. Whenever possible, dedicate one laminar flow hood to culturing and passaging uninfected Sf9 Insect Cells, and a second for all virus work.

1. Place bottle of medium in a 28°C water bath.
2. Retrieve vial of Sf9 Insect Cells and quickly immerse halfway into a 28°C water bath. Swirl gently until cells are fully thawed (~2 min).
3. Sterilize exterior of vial with 70% ethanol and move to a laminar flow hood.
4. Working in a laminar flow hood, carefully open the vial. Slowly pipet cells into a sterile 50-ml polypropylene centrifuge tube (e.g., Falcon™).
5. Add 10 ml pre-warmed 28°C medium drop-wise to the cells. Do not include antibiotics. Return bottle of medium to 28°C. Do not expose cells to this 10 ml dilution medium for more than 2 h, because residual DMSO from frozen stock is harmful during prolonged exposure.

Note: *The medium must be added drop-wise to cells. Do not add cells directly to medium.*

6. Gently pipet cell suspension 3–5 times.
7. Transfer suspension to one sterile T-75 flask. Close the cap. Rock gently to disperse cells evenly.
8. Incubate T-75 flask at 28°C for 30–60 minutes. Cells will attach to bottom of the flask during this period. Do not allow incubation to continue for more than 2 hours.
9. After attachment, gently remove medium with pipet while tipping flask at 45° angle.
10. Pipet 10 ml fresh medium (pre-warmed to 28°C) into the T-75 flask. Medium may contain 5% (v/v) fetal bovine serum, if desired. Gently rock T-75 flask to distribute medium evenly.
11. Continue incubation at 28°C until the monolayer becomes 85–95% confluent. Check for viability and confluency every other day under a microscope. Cells can also be assayed for Trypan blue exclusion (see protocol on p 3).

Passaging Monolayer Cultures

1. Pre-warm bottle of medium to 28°C.
2. Under a laminar flow hood, examine monolayer under a microscope to ensure cells are healthy and confluent (85–95%).

Note: *Healthy Sf9 cells appear rounded with distinct cell boundaries, as compared to unhealthy, granular cells. Numerous floating cells generally signify unhealthy culture. However, Sf9 cells grown beyond confluency will also float and divide, as they are not subject to contact inhibition.*

3. Gently aspirate medium from the flask.
4. Pipet 10 ml fresh medium, pre-warmed to 28°C, into the T-75 flask.
5. Use a sterile scraper or repeated pipetting to gently dislodge cells.Transfer dislodged cells to a sterile 50-ml polypropylene centrifuge tube.
6. Count cells using the Trypan blue exclusion method (p 3).

7. Based on the cell count, seed cells to new flasks according to desired application.

Typically, cells grown at 28°C in a monolayer are split 1:8 every 3–4 days. Depending on needs, nearly confluent monolayer cells can be split at any ratio between 1:2 and 1:20. As more cells are required, additional flasks can be seeded. Typical seeding densities are listed in Table 2.

Table 2

Flask Size	Cell Number	Medium Volume
25-cm^2 flask	1.0×10^6	5 ml
75-cm^2 flask	3.0×10^6	10 ml
150-cm^2 flask	6.0×10^6	30 ml

Passaging Suspension Cultures

Sf9 Insect Cells can be grown and maintained in suspension. Exponentially growing cells are incubated in a temperature-controlled orbital shaker operating at 28°C at 150 rpm. For proper aeration, total culture volume should not comprise >20% of the flask volume, and threaded caps should be kept slightly loose.

1. Follow Steps 1–5 of the *Passaging Monolayer Cultures* protocol (above).
2. Under a laminar flow hood, examine a 1-ml aliquot of Sf9 Insect Cells from a dislodged monolayer or prepared suspension culture under a microscope. Determine whether cells are healthy and confluent (85–95%).
3. Count cells using Trypan blue exclusion method (below). Based on the cell count, seed cells to new flasks according to desired application.

Suspension cells are usually seeded at 1×10^6 cells/ml in a total volume of 50 ml in a 250-ml sterile plastic Erlenmeyer flask. To maximize viability and virus or protein production, maintain cells at a concentration between 1×10^5 and 4×10^6 cells/ml. Split cells when density is $3–4 \times 10^6$ cells/ml.

Note: Overdilution will result in cell death; avoid densities less than 0.2×10^6 cells/ml.

Trypan Blue Exclusion Method

1. Add 100 µl cells to 100 µl Trypan blue solution (0.4% Trypan blue in 0.85% saline). Pipet up and down 5 times to mix. Immediately aliquot a small amount of mixture to both sides of a hemocytometer.
2. Use hemocytometer according to manufacturer's instructions to count cells. For improved accuracy, repeat the count using several samples and record the average. Trypan blue dye stains only dead cells. Perform cell counts very soon after the dye is added, as living cells lose their capacity to exclude dye over time.

Preparation of Exponentially-Growing Sf9 Cells for Long-Term Storage

1. Count cells using Trypan blue exclusion method (above) to ensure >90% viability.
2. Adjust cell density to 2×10^7 cells/ml with BacVector® Insect Cell Medium.
3. Prepare an equal volume of freezing medium [BacVector Insect Cell Medium containing 20% (v/v) DMSO and 5% fetal bovine serum (v/v)].

Note: It is important to use high-grade DMSO.

4. Add an equal volume of freezing medium in drop-wise fashion to cells. Gently pipet cell suspension to ensure complete mixing.
5. Aliquot 1 ml cell suspension into each cryogenic vial and close caps tightly. Place vials at –20°C for 2 hours.
6. Transfer vials to –70°C as rapidly as possible. Keep at –70°C for 12–16 hours.
7. Transfer vials to a liquid nitrogen tank as rapidly as possible for long-term storage (up to 6 months).

Reference

1. King, L.A. and Possee, R.D. (1992) *The Baculovirus Expression System: A Laboratory Manual*, Chapman & Hall, UK.