TriEx™ Sf9 Cells

TriEx™ Sf9 Cells	3 vials	71023-3

About the Kit

Description

Novagen® TriEx™ Sf9 Cells are derived from the *Spodoptera frugiperda* cell IPLB Sf21-AE (ATCC CRL-1711) (1). TriEx Sf9 Cells are adapted for vigorous growth, high cell density, and optimal protein expression when used in conjuction with TriEx Insect Cell Medium and pTriEx and pBAC™-derived baculoviruses constructed with BacVector® Triple Cut Virus DNA. The cells are frozen using a protocol that optimizes recovery of viable cells after thawing. It is important to note that TriEx Sf9 Cells and TriEx Insect Cell Medium cannot be used for co-transfection of baculovirus DNA and transfer plasmids to construct recombinant baculoviruses, or for conventional plaque assays using agarose overlays. For these purposes, we recommend Novagen Sf9 Insect Cells and BacVector Insect Cell medium. The TriEx combination is ideal for production of high titer virus stocks and baculovirus infection for protein expression.

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

Components

Sf9 Insect Cells

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

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Storage of Cells Upon Receipt

Immediately upon receiving the shipment, remove TriEx™ 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 TriEx Sf9 Cells* below).

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

Thawing TriEx™ Sf9 Cells

TriEx Insect Cell Medium (Cat. No. 71022-3) is recommended in the following protocols. Serum-free medium is normally recommended, but medium with serum can be used if desired. Antibiotics may be used, except where noted in transfection procedures. Some lots of antibiotics may severely inhibit cell growth. We therefore recommend testing new lots for compatibility with the cells prior to routine use.

1. Place bottle of medium in 28°C water bath. Medium warmed to 28°C is required at Step 6.
2. Retrieve vial of TriEx Sf9 Cells from freezer or liquid N_2 and immediately transfer vial to 37°C water bath.
3. Thaw cells quickly by immersing vial about halfway into 37°C water bath. Gently swirl vial until cells are fully thawed (approximately 2 min).
4. Sterilize exterior of vial with 70% ethanol.
5. Using aseptic technique in a laminar flow hood, carefully open vial. Slowly pipet contents of vial into sterile 50-ml polypropylene centrifuge tube (e.g., Falcon™).
6. Add 10 ml pre-warmed medium drop-wise to cells. Do not include antibiotics. Return bottle of medium to 28°C. Do not expose cells to this initial 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 the medium.

7. Gently pipet cell suspension 3–5 times. Transfer entire contents into one T-75 flask.
8. Gently rock medium with cells in T-75 flask to ensure even dispersion of cells.
9. Close cap tightly and incubate flask at 28°C for 30–60 min. During this incubation, cells will attach to bottom of flask. Proceed directly to Step 10, ensuring that total exposure time to the initial 5 ml medium does not exceed 2 h.
10. After attachment, gently remove medium with pipet while tipping flask at 45° angle.
11. Immediately replace with 5 ml fresh medium pre-warmed to 28°C by pipetting at the side of flask and allowing medium to flow gently across cells.
12. Gently rock T-75 flask to ensure even distribution of medium.
13. Incubate at 28°C until monolayer becomes 80–90% confluent. Check flask every other day to monitor confluency.
14. At this point, initiate shake culture for subsequent virus titering (FastPlax™ Titer Kit, Cat. No. 70850), generation of high titer virus stocks, and protein production. To passage cells, dislodge by pipetting medium from flask over cells. Count cells and dilute with 28°C medium to final concentration of 0.5×10^6 cells/ml. Transfer to suspension culture.

Trypan Blue exclusion method

1. Add 200 µl cells (from Step 7 of *Thawing TriEx™ Sf9 Cells*) to 200 µl Trypan blue solution (0.4% Trypan blue in 0.85% saline). Pipet up and down 5 times to mix.
2. Immediately aliquot a small amount of mixture to both sides of a hemocytometer.
3. 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. However, it is important to perform cell counts very soon after dye is added, as living cells lose their capacity to exclude the dye over time. TriEx™ Sf9 Cells should contain >80% live cells by Trypan Blue exclusion.

Cell Growth and Maintenance of Suspension (Shake) Cultures

TriEx Sf9 Cells are most conveniently grown and maintained in exponential growth using shake flasks and employing serum-free medium and a temperature-controlled orbital shaker operating at 150 rpm and 28°C. Serum-free medium is normally recommended, but medium with serum can be used if desired. We recommend 28°C for standard growth conditions, although lower temperatures may be used with correspondingly increased times for cell and virus replication as well as protein production. CO_2 is not required, due to the composition of TriEx Insect Cell medium. To ensure proper aeration, the liquid culture should comprise no more than 20% of the vessel volume, and threaded caps should be slightly loose.

We routinely grow cells at an intermediate scale (20–200 ml) in sterile disposable plastic or glass Erlenmeyer flasks and infect or passage them when they are in an exponential growth phase (4×10^6 cells/ml). Shaker cultures are passaged every 2–3 days with doubling times of 24 h under the conditions described here. For passaging, count cells and dilute to 0.5×10^6 cells/ml using fresh medium prewarmed to 28°C. Overdilution will cause cell death. In general, maximum densities attained by shake cultures are $5–6 \times 10^6$ cells/ml. However, at these densities the cells stop growing and die rapidly due to accumulation of toxic by-products and lack of oxygen. For maximum viability and successful virus and protein production, maintain cell concentration between 2×10^5 and 5×10^6 cells/ml. For detailed protocols for pTriEx virus and protein production, see User Protocol TB250.

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

Exponentially-growing TriEx Sf9 cells can be prepared using the procedure described in Steps 1–8 below and then stored in liquid N_2 for up to 6 months.

1. Count cells using Trypan blue exclusion method (see protocol on p 2) to ensure >90% viability.
2. Adjust cell density to 2×10^7 cells/ml with TriEx Insect Cell Medium.
3. Prepare an equal volume of freezing medium [TriEx 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.
5. Gently pipet cell suspension to ensure complete mixing.
6. Aliquot 1 ml cell suspension into each cryogenic vial and close caps tightly. Place vials at –20°C for 2 h.
7. Transfer vials to –70°C as rapidly as possible. Keep at –70°C for 12–16 h.
8. Transfer vials to a liquid nitrogen tank as rapidly as possible for long-term storage.

Reference

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