ExpiSf™ Expression System

Smart Start Guide

Pub. No. MAN0017562 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ExpiSf™ Expression System User Guide* (Pub. no. MAN0017532). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ExpiSf™ Expression System protocol

This protocol highlights the typical ExpiSf™ Expression System workflow at 25-mL culture scale starting with transfection of suspension ExpiSf9™ cell culture with bacmid DNA. Please refer to *ExpiSf™ Expression System User Guide* (Pub. No. MAN0017532) for detailed instructions.

Smart Start Tips

Get started quickly with the ExpiSf™ Expression System, and maximize protein yields with these tips for optimal performance. Consult the FAQs in this guide for troubleshooting tips.

- **Shaking**: Refer to the shake grid (Table 1) to ensure that the shaking diameter, shaking speed, and vessel type are optimal for the volume of culture to be transfected/infected.
- **Cell preparation**: Subculture high-density ExpiSf9™ cells when they have reached log-phase growth (e.g., 5×10^6–10×10^6 viable cells/mL). Subculturing ExpiSf9™ cells before or after log-phase growth can negatively impact performance.
- **Bacmid DNA transfection**: We recommend using the suspension-based transfection protocol for baculovirus generation. This will allow you to rapidly produce a large scale, high-quality, high-titer P0 virus to be used directly in downstream protein expression applications. No virus amplification is required. For optimal transfection of suspension ExpiSf9™ cells using ExpiFectamine™ Sf Transfection Reagent, mix by tube inversion. Do not vortex as this will damage bacmid DNA and reduce transfection efficiency and overall baculovirus titers. Also, follow the recommended 5 minute bacmid DNA-lipid complexation time. Longer incubation times may reduce P0 viral titer production.
- **Baculovirus quality**: Amplifying baculovirus past three cycles is not recommended as this will result in reduced protein titer output due to formation of defective interfering viral particles leading to low-quality virus. We recommend following the suspension-based transfection protocol to obtain large volumes of non-amplified P0 virus. If virus amplification is required, it is important to harvest cells at 60–80% viability at each amplification cycle. Do not let cell viability drop below 60% as this may reduce the quality of your baculovirus stock.
- **Cell viability**: It is important to use a healthy cell culture in your experiments. Using cells with < 90% viability may impact the performance of the system and yield sub-optimal results.

Scalability at a glance

The ExpiSf™ Expression System can be easily scalable in protein expression experiments from 24 deep-well plate to 2-L shake flask scale.

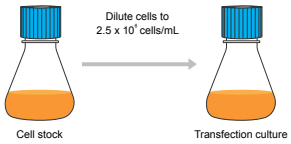
Table 1 Recommended volumes for protein expression using the ExpiSf™ Expression System at different scales. Numbers provided are on a per-shake flask or per-well basis.

Vessel type	24 deep-well plate	125-mL	250-mL	500-mL	1-L	2-L
Total number of cells	20×10^6 cells	1.25×10^8 cells	2.5×10^8 cells	5×10^8 cells	10×10^8 cells	2×10^9 cells
Final viable cell density	5×10^6 cells/mL					
Initial culture volume	4 mL	25–30 mL	50–60 mL	100–120 mL	200–240 mL	400–480 mL
ExpiSf™ Enhancer	16 µL	100 µL	200 µL	400 µL	800 µL	1.6 mL
Volume of baculovirus stock[1]	40–80 µL	250–500 µL	500–1000 µL	1–2 mL	2–4 mL	4–6 mL
Recommended MOI[2]	5					
Shaker speed	250 rpm (19-mm shaking diameter)	125 ±5 rpm (19-mm shaking diameter) 125 ±5 rpm (25-mm shaking diameter) 95 ±5 rpm (50-mm shaking diameter)				
Recommended flask type	—	PETG, sterile, non-baffled, vented shake flask				

[1] Recommended starting volumes of P0 baculovirus stock to use if virus titer was not determined.
[2] If virus titer was determined.

Recombinant baculovirus production

1 Day 1: Prepare cells for transfection



a. At the time of transfection, dilute cells to 2.5×10^6 cells/mL (≥ 90% viability) in 25 mL ExpiSf™ CD Medium in a 125-mL PETG, sterile, non-baffled, vented shake flask.

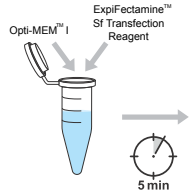
b. Use the following steps to prepare the cells:

1. Pipet 62.5×10^6 viable cells into a sterile 50-mL conical tube.
2. Centrifuge at 300 × *g* for 5 minutes.
3. Aspirate the supernatant and gently resuspend cells in 25 mL fresh ExpiSf™ CD Medium.
4. Transfer the cell suspension to a 125-mL shake flask.

c. Incubate cells for 0–30 minutes in a 27°C non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform (see "Scalability at a glance" on page 2 for recommended shake speeds for different shaking diameters).

Note: It is important to proceed to Step 2 within 30 minutes of cell seeding. Incubating cells for longer than 30 minutes prior to transfection may result in decreased transfection efficiency.

2 Day 1: Dilute ExpiFectamine™ Sf Transfection Reagent in Opti-MEM™ I Reduced Serum Medium



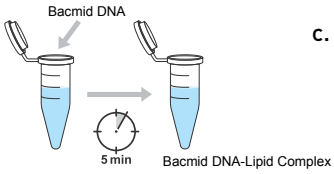
a. Gently mix the ExpiFectamine™ Sf Transfection Reagent before use by inverting the tube 5–10 times.

b. Dilute 30 µL ExpiFectamine™ Sf Transfection Reagent in 1 mL Opti-MEM™ I Reduced Serum Medium.

c. Mix by gently inverting 5–10 times.

d. Incubate the mixture at room temperature for 5 minutes.

3 Day 1: Add bacmid DNA to diluted ExpiFectamine™ Sf Transfection Reagent

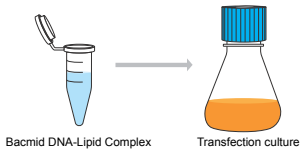


a. Add 12.5 µg bacmid DNA directly to the diluted ExpiFectamine™ Sf Transfection Reagent.

b. Mix by gently inverting 5–10 times.

c. Incubate the mixture at room temperature for 5 minutes.

4 Day 1: Add DNA-lipid complex to cells



a. Slowly transfer the DNA-lipid complex drop-wise to the 125-mL shake flask from Step 1, swirling the flask during addition to ensure uniform delivery.

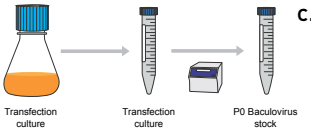
b. Incubate cells in a 27°C non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform (see "Scalability at a glance" on page 2 for recommended shake speeds for different shaking diameters).

Note: ExpiFectamine™ Sf Transfection Reagent exhibits low cytotoxicity; therefore, no media change is required post-transfection.

5 **Day 3–4**: Collect P0 baculovirus

a. Transfer the transfection to a collection tube.

b. Centrifuge at 300 × *g* for 5 minutes.



c. Collect clarified, virus-containing supernatant into a new, clean tube. This is your P0 baculovirus stock.

6 **Day 4–5**: Titer P0 baculovirus

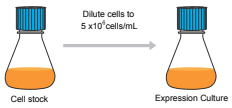
a. Titer P0 baculovirus stock using FACS-based virus titering assay or other method of choice.

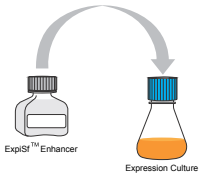
Refer to *ExpiSf™ Expression System User Guide*, Pub. No. MAN0017532 for detailed FACS-based virus titering protocol.

b. Calculate virus concentration (i.e., infectious virus particle (ivp)/mL or other appropriate unit of measure).

Protein expression

1 **Day 5:** Prepare cells for virus infection





a. Ensure that cell viability is ≥90%.

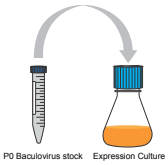
b. Seed ExpiSf9™ cells at 5 × 10^6 viable cells/mL in 25 mL ExpiSf™ CD Medium in a 125-mL PETG, sterile, non-baffled, vented shake flask.

c. Immediately after cell seeding, add 100 µL ExpiSf™ Enhancer to the cell culture.

Note: there is no need to pre-warm the ExpiSf™ Enhancer to room temperature prior to addition. Cold reagent can be used.

d. Incubate cells in a 27°C non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform (see "Scalability at a glance" on page 2 for recommended shake speeds for different shaking diameters).

2 **Day 6:** Infect cells



a. 18–24 hours after ExpiSf™ Enhancer addition, infect cells with P0 baculovirus stock at MOI of 5.

Note: If virus titer was not determined, we recommend infecting cells using 250–500 µL P0 baculovirus stock.

b. Incubate cells in a 27°C non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform (see "Scalability at a glance" on page 2 for recommended shake speeds for different shaking diameters).

3 **Day 7–10:** Harvest cells and/or media

Harvest cells or media (if recombinant protein is secreted) and isolate protein using your method of choice.

Frequently asked questions

Where can I find additional information to help me get started using the ExpiSf™ Expression System?

See **thermofisher.com/ExpiSf** for comprehensive product information.

How should the ExpiSf9™ cells be handled upon receipt?

Upon receipt of the cells on dry ice, it is best to either thaw the cells immediately or place the vials into liquid nitrogen storage for approximately 72 hours to allow cells to acclimate before proceeding with thaw.

IMPORTANT! Do not store cells at −80°C.

Thaw cells into room temperature media using a non-baffled, vented shake flask and incubate cells in a 27°C non-humidified, non-CO_2 atmosphere incubator on a shaker platform set to 125 ±5 rpm for shakers with a 19-mm or 25-mm shaking diameter or 95 ±5 rpm for shakers with a 50-mm shaking diameter. Cells should have high viability at the time of thaw and should recover quickly post-thaw, reaching their normal 24 hour doubling time within 1–2 passages.

What are the normal growth characteristics of ExpiSf9™ cells?

Within 1–2 passages post-thaw, ExpiSf9™ cells should have a doubling time of approximately 24 hours. When cells are cultured at 0.5×10^6 viable cells/mL or 1.0×10^6 viable cells/mL, viable cell density should be approximately 5×10^6–10×10^6 cells/mL within 4 or 3 days, respectively. If cells are not growing within these approximate ranges, further optimization of cell culture conditions are needed.

Why are my cells not growing post-thaw?

Verify that the culture volume and shake speed are set according to Table 1.

What are the best practices for handling ExpiSf9™ cells?

ExpiSf9™ cells do not reach log phase growth until approximately 4×10^6 cells/mL, thus ExpiSf9™ cells should be allowed to attain a density of 5×10^6–10×10^6 viable cells/mL before subculturing to ensure the cells have reached log phase growth. For all cell manipulations, swirl flasks to resuspend the cells. Do not shake or pipet the cells vigorously to mix, as this can lead to decreased performance, especially just prior to transfection and virus infection.

How soon after thawing can I use my cells and how many passages are they good for?

ExpiSf9™ cells should be passaged at least two times post-thaw and be growing within the ranges specified in the product manual, prior to transfection and/or viral infection. Cells should maintain consistent performance for 25–30 passages if maintained in accordance with the cell culture maintenance guidelines in the manual.

What if my cells overgrow the 5×10^6–10×10^6 viable cells/mL target density ahead of subculturing?

If cells significantly overgrow the target of 5×10^6–10×10^6 viable cells/mL (i.e. growing above 10×10^6 viable cells/mL), subculture cells at a density of 0.5×10^6–1.0×10^6 viable cells/mL and continue to passage them a couple of times to allow the cells to recover. Monitor cell viability and growth kinetics to ensure cells are healthy (i.e., ≥90% viability and reaching 5×10^6–10×10^6 viable cells/mL at Day 3–4 post-passaging).

How can I ensure that my cells are growing optimally?

As a quick check, seed ExpiSf9™ cells at 0.5×10^6 cells/mL in 25 mL of ExpiSf™ CD Medium in a 125-mL non-baffled, vented, shake flask and monitor cell viability and viable cell density of the culture everyday, or at least on days 5, 6, 7 post-seeding. Typically, ExpiSf9™ cells will reach maximal density of 18×10^6–20×10^6 cells/mL around Day 7–8 post-seeding. The cells will continue to show high viability for a few days (e.g., Day 7–10), after which viability will drop.

Note: If cells exhibit significantly different growth profiles, optimize culture conditions. Test multiple different shaker speeds simultaneously to determine which speed provides optimal cell growth and then use this shaker speed for your experiments.

Can I substitute other media for ExpiSf™ CD Medium?

No. The ExpiSf™ Expression System is optimized for maximal protein expression and achieves high titers due to the fact that all components work together for optimal performance. ExpiSf™ CD Medium is a chemically defined, yeastolate-free, animal origin-free growth media specifically matched to ExpiSf™ Enhancer. ExpiSf™ CD Medium is also essential to support high-density growth of ExpiSf9™ cells and enable high-density infections.

What density should the ExpiSf9™ cells be at the time of transfection?

The ExpiSf™ Expression System allows you to rapidly generate high-titer baculovirus using a suspension-based protocol. ExpiSf9™ cells should be seeded at a density of 2.5×10^6 viable cells/mL just prior to transfection. After seeding, cells are returned to the incubator for 0–30 minutes and then transfected following the recommended protocol.

If desired, ExpiSf9™ cells can also be transfected in adherent culture format. When using adherent ExpiSf9™ cell culture, cells should be seeded at a density of 1×10^6 cells/well in 6-well plates just prior to transfection when using the suspension protocol. After seeding, cells are returned to the incubator for 30–60 minutes to allow them to adhere to the plate and then transfected following the recommended protocol.

Do I really need to titer my baculovirus?

As best practice, we recommend titering your baculovirus stock prior to protein expression experiments. If you choose not to titer your virus stock, we recommend using 250–500 µL of P0 virus stock for protein expression at the 25 mL culture scale. However, for optimal protein expression, a titration of your virus stock may be required to determine the optimal amount to be used in your infections.

What are the recommendations for properly storing my baculovirus stocks?

Baculovirus stocks can be stored at 4°C protected from light for up to 12 weeks. Alternatively, baculovirus stocks can be frozen and stored at −80°C or in liquid nitrogen for longer periods. We do not recommend repeated freeze/thaw cycles of your virus stock. Frozen virus should be stored in small aliquots and not re-frozen once thawed. Following these guidelines, baculovirus stocks can be stored without DMSO or cryopreservatives (e.g., FBS or BSA). If virions will be stored for longer than 12 weeks, adding 0.1% to 1% BSA to stabilize the virus is recommended. Store the virus stocks in polypropylene containers or siliconized glassware to prevent nonspecific binding of virus. Virus stocks should be re-titered periodically if used as inoculates.

What baculovirus expression vector system (BEVS) do you recommend using with this system?

The ExpiSf™ Expression System has been optimized with the Bac-to-Bac™ Baculovirus Expression System. Although other BEVS technologies may be used with the ExpiSf™ Expression System, experimental conditions should be established empirically.

When should the ExpiSf™ Enhancer be added to the ExpiSf™ cell culture?

Add ExpiSf™ Enhancer at the time of cell seeding, 18–24 hours prior to virus infection for best results. This solution may be added to the culture without pre-warming.

What density should the ExpiSf9™ cells be at the time of virus infection?

One day prior to virus infection (Day −1), ExpiSf9™ cells should be seeded at a density of 5×10^6 viable cells/mL and immediately treated with ExpiSf™ Enhancer. The next day (Day 0; 18–24 hours after ExpiSf™ Enhancer addition), cells should be at a density of 5×10^6–7×10^6 viable cells/mL and ready for infection. It is important to infect cells within the 18–24 hours period post-Enhancer treatment, for optimal performance.

Note: Incubating ExpiSf™ Enhancer-treated cells for longer than 28 hours may result in decreased infection efficiency and low protein titers.

What if my cells are at a density greater than 7×10^6 cells/mL at the time of virus infection?

We have observed a decrease in infectivity when cell densities are greater than 7×10^6–8×10^6 viable cells/mL at time of infection. If cell density is higher than 7×10^6–8×10^6 viable cells/mL 18–24 hours after addition of ExpiSf™ Enhancer, it is likely that the initial cell seeding density at Day −1 may have been above 5×10^6 viable cells/mL. Verify your cell counts using an alternative method, such as trypan blue exclusion using a hemacytometer, to ensure that cells are being accurately counted and seeded for your experiments.

How can I tell if my cells have been efficiently infected with virus?

When ExpiSf9™ cells are efficiently infected, viability will decrease to 60–80% at Day 3 post-infection. In parallel, viable cell density should be between 5×10^7–7×10^7 cells/mL and cell diameter will increase from 16 µm (uninfected) to 18–20 µm. Finally, observing under a microscope, infected cells will have enlarged nuclei and the cytoplasm may contain vacuoles and demonstrate granularity.

What day should I harvest my supernatant/pellet?

Due to the lytic nature of the baculovirus expression system, infected cells will eventually lyse. Therefore, the time of harvest should be determined empirically and is highly dependent on the nature of your protein as well as the infection kinetics. As a general rule, 48–96 hours post-infection is a commonly-used harvest time range.

Limited product warranty

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