Adaptation of the ExpiSf Expression System for high-level protein production with the WAVE Bioreactor System

Introduction

The Gibco™ ExpiSf™ Expression System is a complete, chemically defined baculovirus–insect cell system for expression of recombinant proteins that is designed to deliver superior yields and consistent performance using a fast, streamlined workflow. The standard protocol used with the ExpiSf system provides a robust method that is optimized for cultures maintained in standard culture flasks. However, the flexibility of the system enables the use of high-volume culture formats for bulk production.

Here, we present a method for the adaptation of the ExpiSf system for use in the WAVE Bioreactor™ System (GE Healthcare) at the 10 L scale. We show that the Cellbag™ Bioreactor Chambers that are used with the WAVE Bioreactor System maintain high protein yields, which enables a seamless transition from culture flasks to the Cellbag bioreactor format. Because mixing, gas exchange, and shear stresses in the WAVE Bioreactor System are different compared to standard culture flasks, optimization of culture conditions for different proteins may be required depending on bag size and fill volume. This application note describes general conditions for the expression of three different recombinant proteins.

Materials

For one 10 L run:

- Gibco™ ExpiSf9™ Cells (Cat. No. A35243)
- Gibco™ ExpiSf™ Protein Production Kit, for 10 L of culture (Cat. No. A3767807)
- Gibco™ Bac-to-Bac™ Baculovirus Expression System (Cat. No. 10359016)
- Cellbag™ 22 L (Oxywell Version) Bioreactor Chamber (GE Healthcare, Cat. No. CB0022L10-02)
- ReadyToProcess™ WAVE 25 Rocker (GE Healthcare, Cat. No. 28988000) or comparable unit such as Thermo Scientific™ Finesse™ SmartRocker™ Bioreactor System (Thermo Fisher Scientific) or BIOSTAT™ RM Bioreactor (Sartorius)

Methods

1. **Prepare cells (day –8 to –7):** Grow and maintain ExpiSf9 cells in shake flasks as directed in the ExpiSf Expression System user guide (Pub. No. MAN0017532). Three to four days prior to inoculation of the Cellbag bioreactor, prepare seed culture by expanding ExpiSf9 cells to a density of 5 x 10^6–10 x 10^6 viable cells/mL (≥90% viability) in 500–1,000 mL of culture volume. Note: For these experiments, a total of 5 x 10^9 viable cells is needed per Cellbag bioreactor.

2. **Prepare the WAVE system and pre-equilibrate culture medium (day –5):** For these experiments, a Cellbag bioreactor rated to contain up to 10 L of culture was used. For a 10 L final culture volume, approximately 4 L of initial medium is required. One day prior to inoculation, aseptically connect a 10 L bag of Gibco™ ExpiSf™ CD Medium (part of Cat. No. A3767807) to the 22 L Cellbag bioreactor. Assemble and prepare the Cellbag bioreactor on the rocker platform of the WAVE Bioreactor System, making sure to smooth out any folds prior to pressurization. Connect the heating element to the outgassing filter. Connect the gas line to the gas input filter port on the bag, and partially inflate the Cellbag bioreactor at a pump rate of 0.5 L/min. After the Cellbag bioreactor is inflated, fill the Cellbag bioreactor with 4 L of ExpiSf CD Medium. After filling, clamp off the tubing at the Cellbag bioreactor and at the medium bag but leave the connections intact. Set the rocker platform to an 8° angle and a rocking speed of 18–20 rpm. Set the temperature to 27°C, gas flow rate to 0.3 L/min, and dissolved oxygen to 30%. Install cover, as this will be required to protect the ExpiSf9 culture from light. Pre-equilibrate the medium overnight.

3. **Inoculate the Cellbag bioreactor** (**day –4**): On the day of inoculation, ExpiSf9 cells should be at a density of 5 x 10^6–10 x 10^6 viable cells/mL (≥90% viability), as described in step 1. Inoculate the Cellbag bioreactor with a total of 5 x 10^9 viable cells by following these steps: Calculate the volume of seed culture required for 5 x 10^9 total viable cells. Aseptically transfer this volume into the Cellbag bioreactor already containing 4 L of pre-equilibrated ExpiSf CD Medium (from step 2). Bring the total culture volume in the Cellbag bioreactor to 5 L by adding additional fresh ExpiSf CD Medium as necessary from the connected medium bag. The final cell density in the Cellbag bioreactor should be 1 x 10^6 cells/mL. Allow the culture to grow for 72 h or until cells reach a density of 7 x 10^6–10 x 10^6 viable cells/mL.

4. **Prepare cells for infection and add enhancer (day –1)**: One day prior to infection, approximately 72 h after inoculation of the Cellbag bioreactor, determine cell density and viability. Cells should reach a density of 7 x 10^6–10 x 10^6 viable cells/mL at ≥90% viability. Dilute the culture to 5 x 10^6 viable cells/mL by aseptically transferring the appropriate volume of ExpiSf CD Medium from the connected medium bag into the Cellbag bioreactor. Note: The final culture volume after dilution will be 8–10 L depending on how much volume is needed to dilute the cell suspension to the required final density of 5 x 10^6 cells/mL. Clamp off the tubing at the Cellbag bioreactor and at the medium bag after transfer. Then, aseptically transfer 40 mL of Gibco™ ExpiSf™ Enhancer (part of Cat. No. A3767807) into the Cellbag bioreactor. Maintain the rocking speed at 18–20 rpm and incubate the culture overnight (18–24 h).

5. **Infect cells with baculovirus stock (day 0)**: On the day of infection, determine cell density and viability. Cell density should be between 5 x 10^6 and 7 x 10^6 viable cells/mL at ≥90% viability. Calculate the volume of baculovirus stock needed to infect the culture. We recommend using a multiplicity of infection (MOI) of 5 as a starting point. Aseptically transfer the calculated volume of baculovirus stock into the Cellbag bioreactor. Note: An MOI of 5 is consistent with the MOI recommended when using the ExpiSf system at shake flask culture scale. However, the optimal MOI may vary depending on the protein being expressed and may need to be further optimized.

6. **Determine optimal time of harvest** (**day 2 to 5**): The time of peak protein expression and harvest should be determined empirically for each protein; however, for the proteins tested, maximum expression was obtained 72–120 h postinfection, when cell viability dropped to 50–70% and cell diameter increased to 17–20 µm.

Table 1. Summary of adaptation protocol.

Step	Day	Description	Instructions
1	–8 to –7	Prepare cells	Expand ExpiSf9 cells to 5 x 10^6–10 x 10^6 viable cells/mL in 500–1,000 mL volume
2	–5	Prepare the WAVE system and pre-equilibrate culture medium	• Transfer 4 L of ExpiSf CD Medium to Cellbag bioreactor • WAVE system parameters: – 8° rocker angle – 18–20 rpm rocking speed – 27°C temperature – 0.3 L/min gas flow rate – 30% dissolved oxygen
3	–4	Inoculate the Cellbag bioreactor	• Inoculate bioreactor with 5 x 10^9 total viable cells • Adjust bioreactor volume to 5 L (final cell density: 1 x 10^6 cells/mL)
4	–1	Prepare cells for infection and add enhancer	• Dilute culture to 5 x 10^6 viable cells/mL with fresh ExpiSf CD Medium • Treat culture with 40 mL ExpiSf Enhancer
5	0	Infect cells with baculovirus stock	• Infect cells with an MOI of 5 (Note: MOI may need to be optimized for your protein)
6	2 to 5	Determine optimal time of harvest	• Determine best harvest time for protein • Typical parameters to watch for: cell viability of 50–70% and cell diameter of 17–20 µm

Results

The ExpiSf system displays good scalability over a broad range of culture volumes and formats, including the WAVE Bioreactor System. As with other formats, certain parameters must be optimized in order to maintain a high volumetric yield.

For the Cellbag bioreactor (1–10 L rated volume) used in these experiments, a rock angle of 8° and a rocking speed of 18–20 rpm was selected, as those conditions provided optimal mixing while minimizing the generation of foam within the bag (Table 1). Aiming for a final volume of 10 L, a seed culture of 5 L was used. As the cell doubling time for ExpiSf9 cells in the Cellbag bioreactor is consistent with those observed in shake flasks (approximately 24 h), a culture density of approximately 7 x 10^6–10 x 10^6 viable cells/mL on the day before infection could be predicted. Addition of medium brought the final culture density to 5 x 10^6 viable cells/mL, and the final culture volume to approximately 10 L, followed by addition of the ExpiSf Enhancer. Cells were infected with baculovirus 18–24 h after enhancer addition.

Cell culture density, viability, and cell diameter were monitored in the Cellbag bioreactors over the course of the experiments. Similar cell growth kinetics and protein yields were observed in the WAVE system and 125 mL shake flasks, demonstrating that the ExpiSf system is readily adaptable to high-volume protein expression (Figures 1–3).

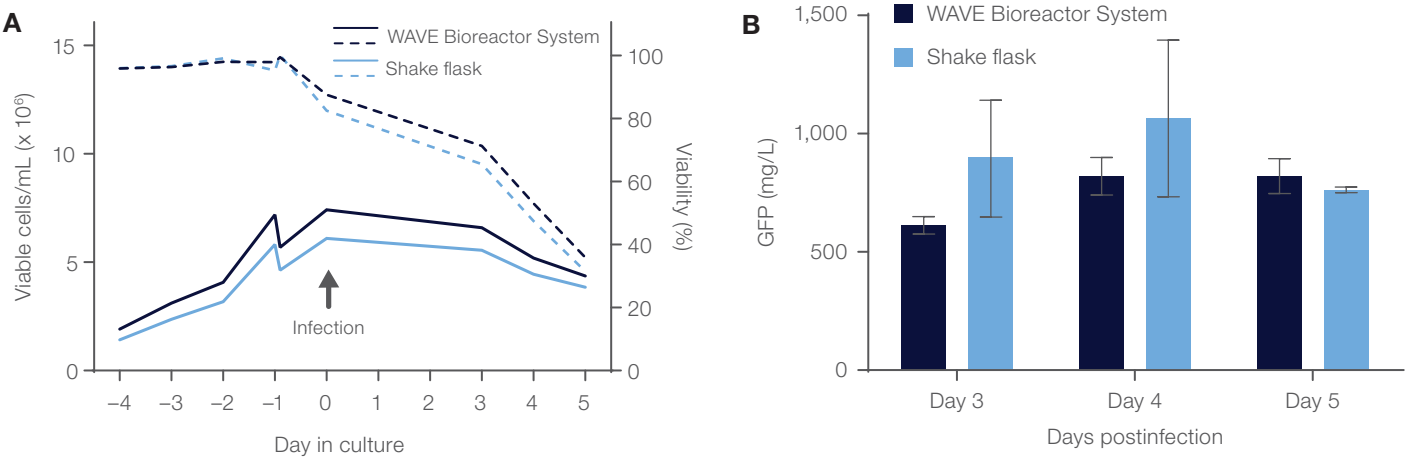

Figure 1. Expression of GFP in the ExpiSf system. (A) Cellular kinetics during the expression run. Solid lines represent viable cell density; dotted lines represent percent viability. **(B)** GFP protein yield. The WAVE Bioreactor System achieved 68%, 77%, and 108% of the control shake flask yields on day 3, 4, and 5 postinfection, respectively.

gibco

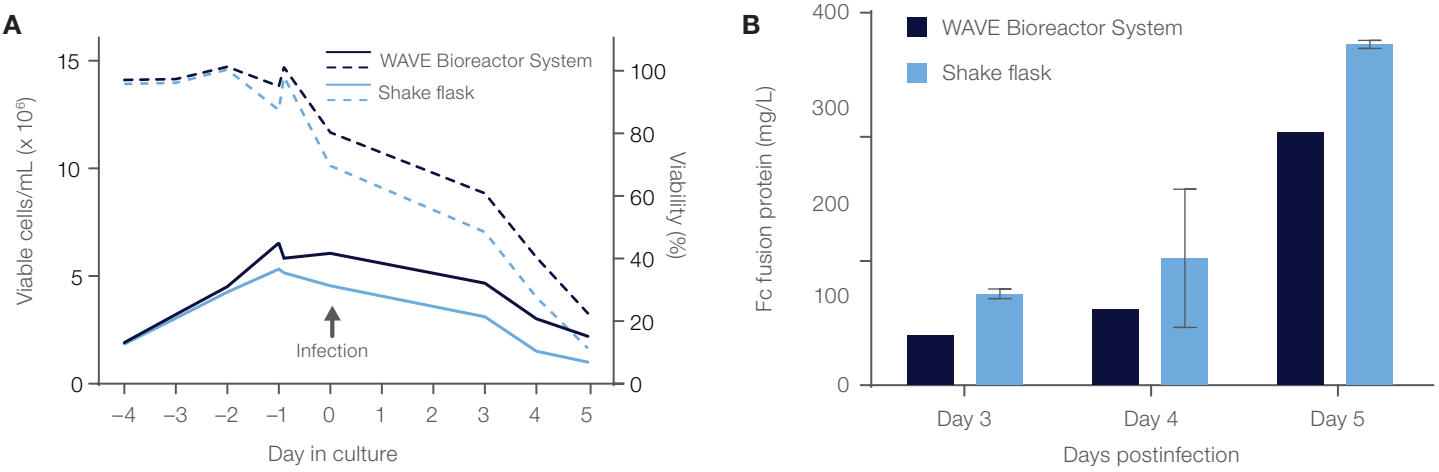

Figure 2. Expression of an Fc fusion protein in the ExpiSf system. (A) Cellular kinetics during the expression run. Solid lines represent viable cell density; dotted lines represent percent viability. **(B)** Fc fusion protein yield. The WAVE Bioreactor System achieved 54%, 60%, and 74% of the control shake flask yields on day 3, 4, and 5 postinfection, respectively.

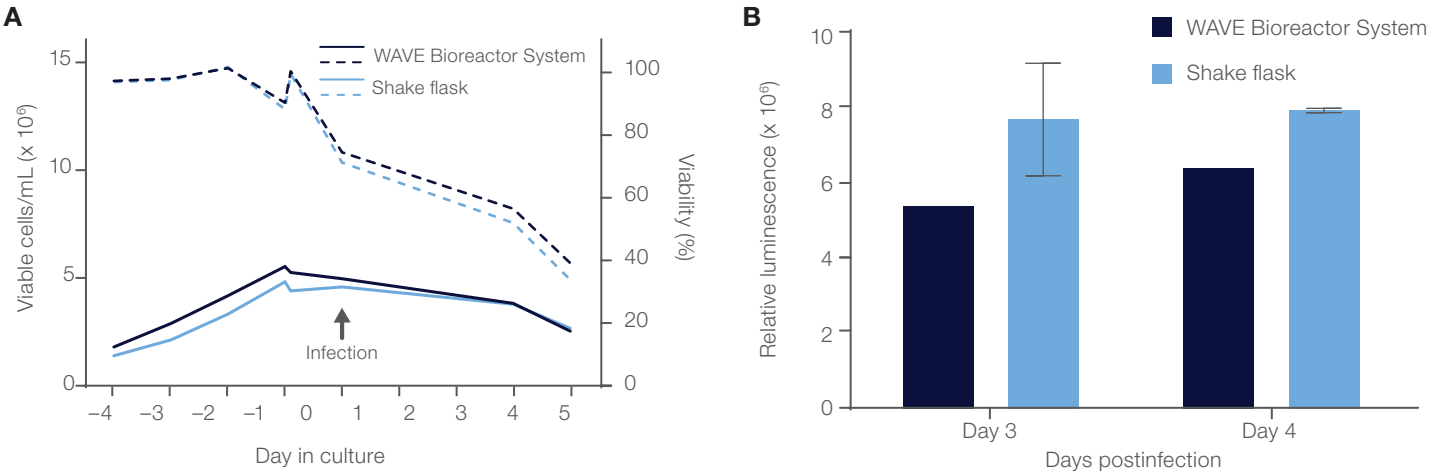

Figure 3. Expression of secreted embryonic alkaline phosphatase (SEAP) in the ExpiSf system. (A) Cellular kinetics during the expression run. Solid lines represent viable cell density; dotted lines represent percent viability. **(B)** SEAP protein yield determined by a luminescence-based enzyme activity assay. The WAVE Bioreactor System achieved 70% and 81% of the control shake flask yields on day 3 and 4 postinfection, respectively.

Conclusion

Here, we present a method for scaling up the ExpiSf Expression System in the WAVE Bioreactor System at the 10 L scale. We show that high protein yields can be achieved, which enables a seamless transition from small-scale shake flask to large-scale Cellbag bioreactor culture format using the ExpiSf system.

Find out more at **thermofisher.com/expisf**

ThermoFisher SCIENTIFIC