

Protein expression

Nalgene 5 L Faceted-Bottom Shake Flask for scale-up of the ExpiSf Expression System

Purpose

This application note describes scale-up of the Gibco™ ExpiSf™ Expression System into the Thermo Scientific™ Nalgene™ 5 L Faceted-Bottom Shake Flask. The unique design of the Nalgene 5 L Faceted-Bottom Shake Flask enhances mixing dynamics, allowing for cell growth and expression levels equivalent to those obtained in a small-scale shake flask.

Introduction

Thermo Scientific™ Nalgene™ 5 L shake flasks are exceptional choices for the culture of suspension cells used in mammalian, insect, and prokaryotic expression systems for the production of recombinant proteins or viral vectors. Our newest additions to the wide range of Nalgene flask offerings are the Nalgene 5 L Angled-Bottom and 5 L Faceted-Bottom Shake Flasks.

Importantly, in addition to the excellent cell growth and protein expression characteristics of cultures grown in Nalgene 5 L flasks, the faceted-bottom version offers improved ergonomic functionality, where the flask may rest at a 45° angle during culture manipulations, helping make for easier pipetting with less strain on the operator.

The ExpiSf Expression System is a complete, chemically defined baculovirus–insect cell expression system that delivers superb protein yields and consistent performance, run after run, 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.



In this study, we compared the growth and protein expression of Gibco™ ExpiSf9™ cells in the Nalgene 5 L Faceted-Bottom Shake Flask to corresponding small-scale shake flask controls. The data indicate that control levels of cell growth and protein expression are obtained with the Nalgene 5 L Faceted-Bottom Shake Flask. The enhanced mixing enabled by the faceted-bottom shake flask is an exceptional choice for ExpiSf9 cells and may also be useful for the scale-up of other robust cell types.

Materials

Materials used for protein expression with Nalgene 5 L Faceted-Bottom Shake Flasks and the ExpiSf Expression System are shown in Table 1. For more details and additional products for baculovirus generation, please see the [ExpiSf Expression System user guide \(Pub. No. MAN0017532\)](#).

Subculture of ExpiSf9 cells

ExpiSf9 cells are capable of achieving high cell densities; therefore, we recommend that the cells attain a minimum density of $5\text{--}10 \times 10^6$ viable cells/mL at the time of subculturing.

Using the viable cell density, the volume of cell suspension required to seed a new shake flask was calculated according to the recommended seeding densities in Table 2 and the recommended culture volumes in Table 3.

Table 1. Materials for protein expression.

Component	Storage	Cat. No.
Nalgene 5 Liter Shake Flask, Faceted Bottom	Room temperature	4115-5001
ExpiSf9 Cells	Liquid nitrogen	A35243
ExpiSf Protein Production Kit, contains: ExpiSf CD Medium ExpiSf Enhancer	2–8°C, protect from light	A3767806

Table 2. Recommended seeding densities for routine cell culture maintenance and infection in Nalgene 5 L Faceted-Bottom Shake Flasks.

Subculture timing	Recommended seeding density
To obtain cells ready at 3 days post-subculture	$0.75\text{--}1 \times 10^6$ viable cells/mL
To obtain cells ready at 4 days post-subculture	$0.4\text{--}0.6 \times 10^6$ viable cells/mL

Table 3. Recommended conditions for routine cell culture maintenance and infection in Nalgene 5 L Faceted-Bottom Shake Flasks.*

Parameter	Condition
Culture volume	2,000–3,000 mL
Protein expression volume	2,000 mL
Target shake speed**	115 rpm (19 mm shaking diameter) 110 rpm (25 mm shaking diameter) 110 rpm (50 mm shaking diameter)

* Refer to the ExpiSf Expression System user guide (Pub. No. MAN0017532) for routine maintenance in smaller shake flask sizes.

** Due to slight differences in shakers, optimal speeds may differ slightly from the target shake speeds shown. Optimize shake speeds to attain maximal protein expression compared to small-scale shake flask controls.

Infecting ExpiSf9 cells to generate recombinant baculovirus

Recombinant baculovirus generation and baculovirus titration were performed as described in the [ExpiSf Expression System user guide \(Pub. No. MAN0017532\)](#).

Infecting ExpiSf9 cells

Preparation of cells for infection in Nalgene 5 L Faceted-Bottom Shake Flasks

1. ExpiSf9 cells were cultured in Nalgene shake flasks as directed in the ExpiSf9 Expression System user guide (Pub. No. MAN0017532) and Tables 2 and 3.
2. Seven days prior to infection (day -7), cells were seeded into 100 mL of culture medium in a Nalgene 500 mL shake flask, to a density of 1×10^6 viable cells/mL, and grown until the cells reached a density of $5\text{--}10 \times 10^6$ viable cells/mL (Figure 1).
3. Four days prior to infection (day -4), cells were seeded into 1 L of culture medium in a Nalgene 2.8 L shake flask, to a density of 1×10^6 viable cells/mL, and grown until the cells reached a density of $5\text{--}10 \times 10^6$ viable cells/mL (Figure 1).
4. One day prior to infection (day -1), cells were seeded into 2 L of culture medium in a Nalgene 5 L Faceted-Bottom Shake Flask to a density of 5×10^6 viable cells/mL (Figure 1).

5. Immediately after seeding, 8 mL of Gibco™ ExpiSf™ Enhancer was added to the shake flask.
6. The culture was incubated overnight in a 27°C non-humidified, air-regulated, non-CO₂ atmosphere incubator on an orbital shaker platform set according to Table 4.

Day 0: infection

7. At 18–24 hours after the addition of ExpiSf Enhancer, viable cell density and viability were determined. Cell density was approximately $5\text{--}7 \times 10^6$ viable cells/mL with $\geq 80\%$ viability.
8. The cells were then infected using a high-titer virus stock ($\geq 1 \times 10^8$ infectious viral particles/mL) at an MOI of 5. If the virus titer has not been determined, we recommend adding 60–120 mL of a high-titer virus stock to the shake flask.
9. Infected cultures were incubated overnight in a 27°C non-humidified, air-regulated, non-CO₂ atmosphere incubator on an orbital shaker platform set according to Table 4.

10. The cultures were harvested at 72 hours post-infection.

Note: The optimal time to harvest protein depends on the specific properties of the protein being expressed and the protocol selected. Typical harvest times can range from 48 to 120 hours post-infection.

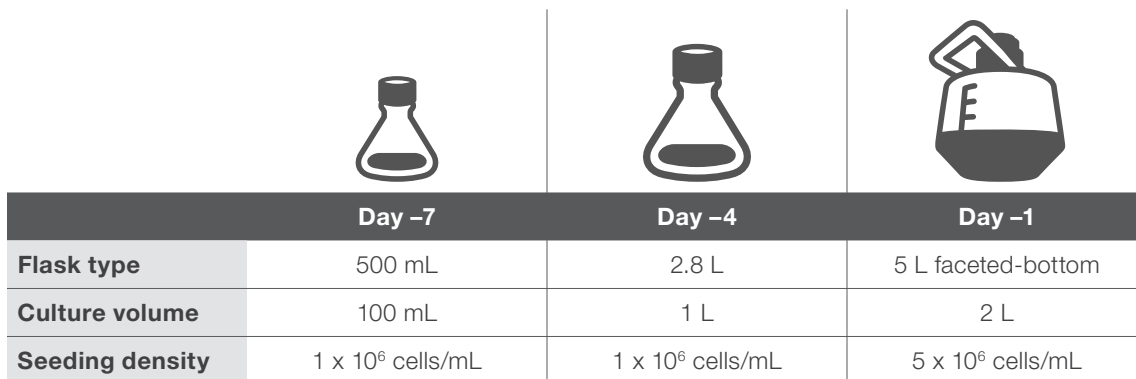


Figure 1. Strategy for scaling up cell cultures prior to infection in Nalgene 5 L Faceted-Bottom Shake Flasks. For these experiments, 10×10^9 viable cells are needed per 5 L shake flask to be infected at a 2 L infection volume.

Table 4. Summary of recommended conditions for infection in Nalgene 5 L Faceted-Bottom Shake Flasks for the ExpiSf Expression System.

Parameter	Condition
Flask size/type	5 L Nalgene Faceted-Bottom Shake Flask
Number of cells required	10×10^9
Culture volume to infect	2 L
Shake speed	115 rpm (19 mm shaking diameter) 110 rpm (25 mm shaking diameter) 110 rpm (50 mm shaking diameter)
Amount of recombinant baculovirus	MOI of 5
ExpiSf9 Enhancer	8 mL
Final culture volume	~2.1 L

Results

Growth of ExpiSf9 cells in Nalgene 5 L Faceted-Bottom Shake Flasks

To assess ExpiSf9 cell growth dynamics in Nalgene 5 L Faceted-Bottom Shake Flasks, ExpiSf9 cells were seeded to a density of 0.5×10^6 viable cells/mL and shaken at 110 rpm on a 25 mm orbital shaker followed by assessment of viable cell density and percent viability over 7 days. Compared to control cultures grown in Nalgene 125 mL flasks, ExpiSf9 cells cultured at either 2 L or 3 L culture volumes in Nalgene 5 L Faceted-Bottom Shake Flasks showed comparable growth dynamics and percent viability (Figure 2).

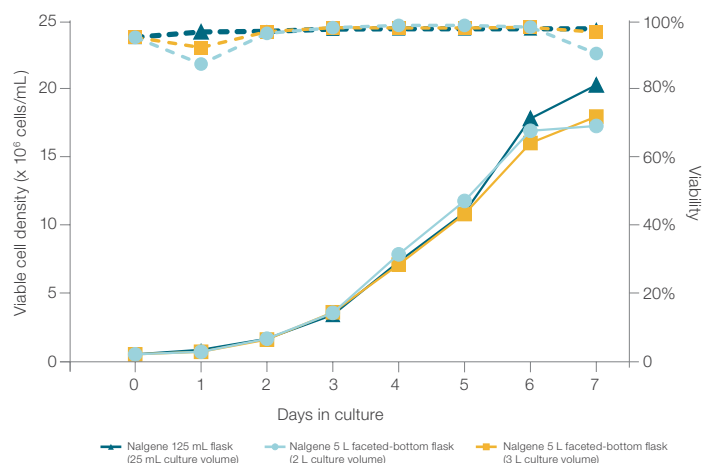


Figure 2. Growth kinetics of ExpiSf9 cells. Viable cell density (solid lines) and viability (dotted lines) for ExpiSf9 cells cultured at 2 L or 3 L final volumes in Nalgene 5 L Faceted-Bottom Shake Flasks are compared to controls cultured in Nalgene 125 mL shake flasks.

Protein expression from ExpiSf9 cells in Nalgene 5 L Faceted-Bottom Shake Flasks

To assess protein expression in Nalgene 5 L Faceted-Bottom Shake Flasks, ExpiSf9 cells were infected according to the method above (steps 7–10) at 5×10^6 viable cells/mL in a volume of 2 L in Nalgene 5 L Faceted-Bottom Shake Flasks. Infected cultures were then incubated for 3 days. Compared to control cultures grown in Nalgene 125 mL flasks, ExpiSf9 cells infected in the Nalgene 5 L Faceted-Bottom Shake Flasks had comparable protein expression (Figure 3).

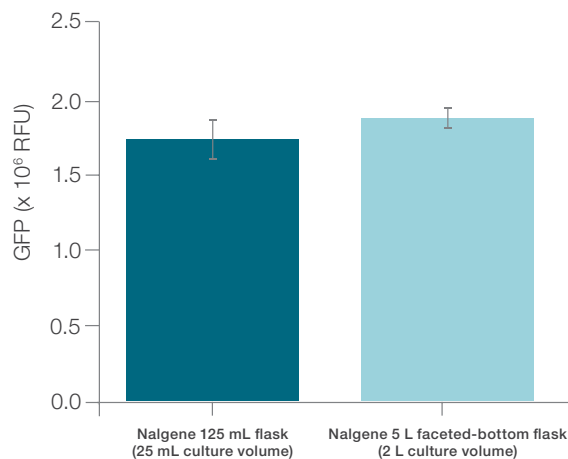


Figure 3. Protein expression levels from ExpiSf9 cells. The protein titer from ExpiSf9 cells infected at a 2 L volume in Nalgene 5 L Faceted-Bottom Shake Flasks was compared to that of Nalgene 125 mL shake flask controls. GFP yields were compared by measuring relative fluorescence units (RFU).

Conclusions

Nalgene 5 L Faceted-Bottom Shake Flasks help enable efficient culture of ExpiSf9 cells to generate maximum protein titers upon infection at a final volume of approximately 2 L of cell culture. Comparable growth and protein expression levels were observed between the Nalgene 5 L Faceted-Bottom Shake Flasks and small-scale control flasks, allowing a seamless transition from small-scale to large-scale shake flask expression formats using the ExpiSf Expression System.

Ordering information

Product*	Quantity	Cat. No.
Nalgene 5 Liter Shake Flask, Faceted Bottom	4/case	4115-5001
ExpiSf9 Cells	1.5 mL	A35243
ExpiSf Protein Production Kit, contains:		
ExpiSf CD Medium	1 L	A3767806
ExpiSF Enhancer	4 x 1 mL	
CO ₂ Resistant Shaker	1	88881101

* For additional products, please see the ExpiSf Expression System user guide (Pub. No. MAN0017532).

 Learn more at thermofisher.com/bigflask

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