Protein expression

Nalgene 5 L Faceted-Bottom Shake Flask for scale-up of the Expi293 Expression System

Purpose

This application note describes scale-up of the Gibco™ Expi293™ 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 Gibco™ Expi293F™ cell growth and expression levels equivalent to those of small-scale shake flasks.

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. The Expi293 Expression System is a mammalian serum-free transient expression system designed to produce high levels of recombinant protein and to scale easily from sub-milliliter to multi-liter formats while maintaining consistent volumetric protein yields.

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. In this study, we compared the growth and protein expression of Expi293F 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 flasks is an exceptional choice for Expi293F cells and may be useful for the scale-up of other robust cell types as well.

Note: For the culture and transfection of Gibco™ ExpiCHO-S™ cells using the Gibco™ ExpiCHO™ Expression System, please refer to the application note "Nalgene 5 L Angled-Bottom Shake Flask for scale-up of the ExpiCHO Expression System". Nalgene 5 L Angled-Bottom Flasks should be used in place of Nalgene 5 L Faceted-Bottom Flasks for protein expression in the ExpiCHO Expression System.

Materials

Materials used for protein expression with Nalgene 5 L Faceted-Bottom Shake Flasks and the Expi293 Expression System are shown in Table 1. For more details, please see the **Expi293 Expression System user guide (Pub. No. MAN0019402)**.

Subculture of Expi293F cells

Expi293F cells are capable of achieving high cell densities; therefore, we recommend that the cells attain a minimum density of 3–5 x 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
Expi293F Cells (1 x 10^7 cells/mL)	Liquid nitrogen	A14527
Expi293 Expression Medium	2–8°C, protect from light	A1435102
ExpiFectamine 293 Transfection Kit, contains: ExpiFectamine 293 Reagent ExpiFectamine 293 Transfection Enhancer 1 ExpiFectamine 293 Transfection Enhancer 2	2–8°C, protect from light	A14525
Opti-MEM I Reduced Serum Medium	2–8°C, protect from light	31985070

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

Subculture timing	Recommended seeding density
To obtain cells ready at 3 days post-subculture	0.4–0.6 x 10^6 viable cells/mL
To obtain cells ready at 4 days post-subculture	0.2–0.4 x 10^6 viable cells/mL

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

Parameter	Condition
Culture volume for cell growth	2,000–3,000 mL
Culture volume to be transfected	1,700 mL*
Target shake speed**	115 rpm (19 mm shaking diameter) 110 rpm (25 mm shaking diameter) 110 rpm (50 mm shaking diameter)
Flask type	Faceted bottom, vented

* For this expression system, it is possible to increase the culture volume to be transfected up to 2.1 L to achieve a final volume of roughly 2.5 L upon addition of transfection complex and enhancers, scaled proportionately.

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

Transfection of Expi293F cells

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

1. Expi293F cells were cultured in Nalgene shake flasks as directed in the Expi293 Expression System user guide (Pub. No. MAN0019402) and Tables 2 and 3.
2. Seven days prior to transfection (day –7), cells were seeded into 220 mL of culture medium in a Nalgene 1 L shake flask, to a density of 0.5 x 10^6 viable cells/mL, and grown until the cells reached a density of 3–5 x 10^6 viable cells/mL (Figure 1).
3. Four days prior to transfection (day –4), cells were seeded into 1 L of culture medium in a Nalgene 2.8 L shake flask, to a density of 0.5 x 10^6 viable cells/mL, and grown until the cells reached a density of 3–5 x 10^6 viable cells/mL (Figure 1).
4. One day prior to transfection (day –1), cells were seeded into 2 L of culture medium in a Nalgene 5 L Faceted-Bottom Shake Flask to a density of 2.5–3 x 10^6 viable cells/mL (Figure 1).
5. On the day of transfection (day 0), cells should have reached a density of approximately 4.5–5.5 x 10^6 viable cells/mL, with viability of 95–99%, appropriate for proceeding with transfection. In a new 5 L faceted-bottom flask, cells were diluted with fresh Expi293 Expression Medium (pre-warmed to 37°C) to a final density of 3 x 10^6 viable cells/mL in 1.7 L total volume, and swirled gently to mix (Figure 1). The cells were then ready for transfection.

Day 0: transfection

6. Complexes of Gibco™ ExpiFectamine™ 293 Reagent with plasmid DNA were prepared as described below. **Note:** The volumes given in steps 6a–6c will generate a complexation reaction sufficient to transfect 1.7 L of culture in a single Nalgene 5 L Faceted-Bottom Shake Flask. Addition of all reagents to this starting volume will result in a final volume of approximately 2 L.
   a. In a 500 mL shake flask, plasmid DNA was diluted by adding 1.7 mL of DNA (assuming a 1 mg/mL plasmid stock) to 102 mL of Gibco™ Opti-MEM™ I Reduced Serum Medium, and mixed by swirling.
   b. The ExpiFectamine 293 Reagent bottle was inverted gently 4–5 times to mix the contents.
   c. In a separate 250 mL shake flask, 5.44 mL of ExpiFectamine 293 Reagent was added to 95.2 mL of Opti-MEM I Reduced Serum Medium, and mixed by swirling.
   d. The two components (diluted plasmid DNA and diluted ExpiFectamine 293 Reagent) were allowed to incubate at room temperature for 3–5 minutes prior to initiating the plasmid DNA complexation reaction.
   e. The diluted ExpiFectamine 293 Reagent was transferred to the diluted plasmid DNA and mixed by gently swirling.

	Day –7	Day –4	Day –1	Day 0
Flask type	1 L	2.8 L	5 L faceted-bottom	5 L faceted-bottom
Culture volume	220 mL	1 L	2 L	1.7 L
Seeding density	0.5 x 10^6 cells/mL	0.5 x 10^6 cells/mL	2.5 x 10^6 cells/mL	3 x 10^6 cells/mL

Figure 1. Strategy for scaling up cell cultures prior to transfection in Nalgene 5 L Faceted-Bottom Shake Flasks. For these experiments, 5.1 x 10^9 viable cells are needed per 5 L shake flask to be transfected at a 1.7 L transfection volume.

7. The ExpiFectamine 293 Reagent/plasmid DNA complex (from step 6e) was allowed to incubate at room temperature for 10–20 minutes, followed by transfer of the complexation mix to the shake flask from step 5, swirling the flask gently during addition.

8. Transfected cultures were incubated in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker (refer to Table 4 for recommended shake speeds) until the addition of enhancers on day 1 post-transfection.

Day 1: enhancer addition

Note: Gibco™ ExpiFectamine™ 293 Transfection Enhancer 1 and ExpiFectamine™ 293 Transfection Enhancer 2 may be premixed together prior to adding to flasks for convenience.

9. On the day after transfection (day 1, 18–22 hours post-transfection), 10.2 mL of ExpiFectamine 293 Transfection Enhancer 1 and 102 mL of ExpiFectamine 293 Transfection Enhancer 2 were added to the flask and mixed by gently swirling the flask during addition. The flask was returned to the 37°C incubator with a humidified atmosphere of 8% CO_2 with shaking.

10. Optimal time to harvest protein will depend on the specific properties of the protein being expressed. 5–7 days post-transfection is a typical harvest time to reach maximum titers for many secreted proteins.

Table 4. Summary of recommended conditions for transfection in Nalgene 5 L faceted-bottom flasks for the Expi293 Expression System.

Parameter	Condition
Flask size/type	5 L Nalgene Faceted-Bottom Shake Flask
Number of cells required	5.1×10^9
Culture volume to transfect	1.7 L
Shake speed	115 rpm (19 mm shaking diameter)
	110 rpm (25 mm shaking diameter)
	110 rpm (50 mm shaking diameter)
Volume of plasmid DNA*	1.7 mL
Opti-MEM medium**	102 mL
ExpiFectamine 293 Reagent	5.44 mL
Opti-MEM medium†	95.2 mL
ExpiFectamine 293 Transfection Enhancer 1	10.2 mL
ExpiFectamine 293 Transfection Enhancer 2	102 mL

* Assuming a plasmid DNA stock concentration of 1 mg/mL and a final concentration of 1.0 µg plasmid DNA per mL of culture volume to be transfected.

** Volume of Opti-MEM medium used to dilute plasmid DNA.

† Volume of Opti-MEM medium used to dilute ExpiFectamine 293 Reagent.

Results

Growth of Expi293F cells in Nalgene 5 L Faceted-Bottom Shake Flasks

To assess Expi293 cell growth dynamics in Nalgene 5 L Faceted-Bottom Shake Flasks, Expi293 cells were seeded to a density of 0.5 x 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, Expi293F 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).

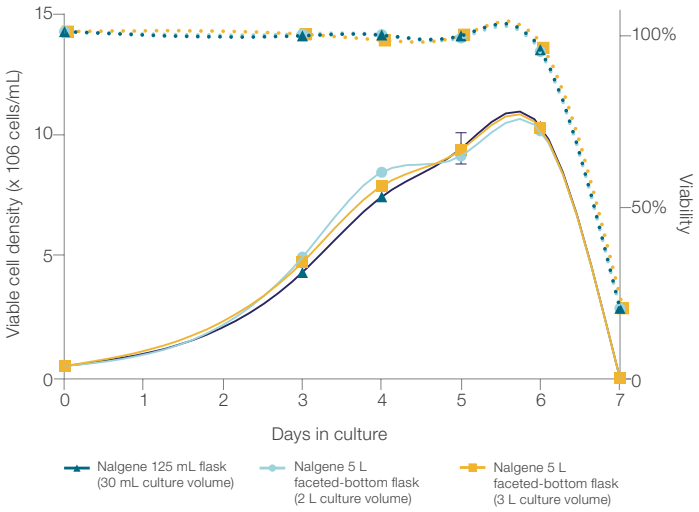

Figure 2. Growth kinetics of Expi293F cells. Viable cell density (solid lines) and viability (dotted lines) for Expi293 cells cultured at 2 L or 3 L volumes in Nalgene 5 L Faceted-Bottom Shake Flasks were compared to controls cultured in Nalgene 125 mL shake flasks.

Protein expression from Expi293F cells in Nalgene 5 L Faceted-Bottom Shake Flasks

To assess protein expression in Nalgene 5 L Faceted-Bottom Shake Flasks, Expi293F cells were transfected according to the method above (steps 6–8) at 3 x 10^6 viable cells/mL in a volume of 1.7 L in Nalgene 5 L Faceted-Bottom flasks. Transfected cultures were then incubated for 6 days. Compared to control cultures grown in Nalgene 125 mL flasks, Expi293F cells transfected in the Nalgene 5 L Faceted-Bottom Shake Flasks had comparable protein expression of a human IgG1 monoclonal antibody (Figure 3).

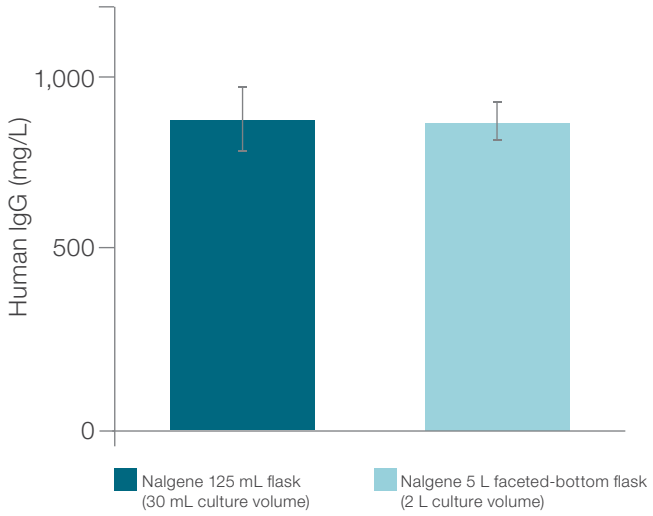

Figure 3. Protein expression levels from Expi293F cells. The protein titer from Expi293F cells transfected at a 2 L volume in Nalgene 5 L Faceted-Bottom Shake Flasks was compared to that of Nalgene 125 mL shake flask controls. Cultures were harvested on day 6 post-transfection to determine protein titers.

Conclusion

Nalgene 5 L Faceted-Bottom Shake Flasks help enable efficient culture of Expi293 cells to help generate maximum protein titers upon transfection in 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 flasks and small-scale control flasks, allowing a seamless transition from small-scale to large-scale shake flask expression formats using the Expi293 Expression System.

Ordering information

Product	Quantity	Cat. No.
Nalgene 5 Liter Shake Flask, Faceted Bottom	4/case	4115-5001
Expi293F Cells (1 x 10^7 cells/mL)	1 mL	A14527
Expi293 Expression Medium	6 x 1 L	A1435102
ExpiFectamine 293 Transfection Kit	For 10 L of culture	A14525
Opti-MEM I Reduced Serum Medium	500 mL	31985070
CO_2 Resistant Shaker	1	88881101

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