Protein expression

Optimizing alternative vessel formats for transient protein production in the Expi293 PRO Expression System

Purpose

This application note describes optimized protocols for transient protein expression with the Gibco™ Expi293™ PRO Expression System using alternative vessel formats not defined in the user guide. These alternative formats enable protein expression from milliliter to multi-liter scales to generate sufficient material for downstream applications while minimizing excess culture volume, resulting in improved efficiency, flexibility, and cost-effectiveness.

Introduction

The Expi293 PRO Expression System is a fully integrated, high-performance expression platform designed to deliver higher protein yields—including for challenging or traditionally low-yield targets—with exceptional speed and reliability.

This expression system is highly adaptable and can be successfully implemented across multiple vessel formats. This application note outlines the tested vessel options, provides ordering information, and details the optimized protocols developed to help ensure consistent performance across these alternative formats.

Materials

The Expi293 PRO Expression System consists of high-performing Expi293 PRO Cells, chemically defined and animal origin–free medium, a complete transfection kit, and plasmid DNA complexation buffer (Table 1). Expression vessels evaluated with these components are listed in Table 2.

Table 1. Components of the Expi293 PRO Expression System.

Item	Amount	Cat. No.
Gibco™ Expi293™ PRO Cells	1 vial	A40001140
	6 vials	A40001978
Gibco™ Expi293™ PRO Expression Medium	1 L	A4000135501
	6 x 1 L	A4000135505
	10 L	A4000135503
	20 L	A4000135504
Gibco™ Expi293™ PRO Transfection Kit	1 L kit	A40002926
	6 x 1 L kit	A40002929
	10 L kit	A40002927
	5 x 10 L kit	A40002930
Gibco™ Opti-Plex™ Complexation Buffer	100 mL	A4096801
	500 mL	A4096803

Table 2. Expression vessels tested in this study.

Type of vessel	Supplier	Cat. No.	Additional materials
Extra-high 24 deep-well plates	EnzyScreen	CR1424hd	Plate cover: Cat. No. CR2224a Clamping system: Cat. No. CR1603h
6 deep-well plates	EnzyScreen	CR1406	Sandwich cover: Cat. No. CR2206a Clamping system: Cat. No. CR1603h
	Thomson Instrument Company	NC3833616	NA
Thermo Scientific™ Nunc™ 50 mL Bioreactor Tubes	Thermo Fisher Scientific	332260	Tube rack: Cat. No. 102182 (Kuhner)
TubeSpin™ 600 mL Bioreactor Tubes	MidSci	TP87600	Tube rack: Cat. No. TP99013 (MidSci) Platform for Infors™ Multitron™ Shaker: Cat. No. TP87631 (MidSci)
7 L shake flasks	Thomson Instrument Company	NC2950617 (fishersci.com)	NA

Methods

Optimizing culture conditions for different types of culture vessels

When optimizing conditions for a new expression vessel, we recommend first testing shake speeds and working volumes to determine the optimal combinations for cell growth. This can typically be accomplished by seeding cells in various culture volumes, and shaking at various speeds, followed by determination of viable cell density and percent viability on multiple days post-cell inoculation. For example, to optimize culture conditions for 6 deep-well plates, Expi293 PRO Cells were added to each well at a final density of 0.5 x 10^6 viable cells/mL, in a final volume of 20, 30, or 40 mL, and shaken at 210, 225, and 240 rpm on shakers with an orbital diameter of 25 mm. Viable cell density and percent viability were determined daily on days 4, 5, and 6 of the growth curve and compared to conditions of a 125 mL control shake flask. The volume and speed conditions that most closely resembled the growth characteristics of the 125 mL control shake flasks were then chosen to test for expression in the 6 deep-well plate.

Overview of conditions for the Expi293 PRO Expression System

Growth and transfection of Expi293 PRO Cells was performed according to the user guide of the Expi293 PRO Expression System (documents.thermofisher.com/TFS-Assets/LSG/manuals/MAN1001709-Expi293PROExpressionSystem-UG.pdf).

Briefly, Expi293 PRO cell density was adjusted to 5 x 10^6 viable cells/mL at the time of transfection. Cultures were incubated in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker of either 25 or 50 mm diameter.

During the complexation step, 100 µL of Opti-Plex Complexation Buffer (10% of culture volume), 1.0 µg of DNA, and 4.5 µL of Expi293 PRO Transfection Reagent (0.45%) were added per mL of culture to be transfected.

One day post-transfection, cultures were supplemented with 80 µL of Expi293 PRO Feed (8%) and 6 µL of Expi293 PRO Enhancer (0.6%) per mL of culture transfected. For each expression run of alternative vessel formats, 125 mL shake flasks served as controls. For general reference, see Table 3.

Table 3. Component amounts used in expression runs.

Component	Amount
Expi293 PRO Cells	5 × 10^6 viable cells/mL density at transfection
Opti-Plex Complexation Buffer	100 µL per mL of culture volume to be transfected (10%)
Plasmid DNA	1.0 µg plasmid DNA per mL of culture volume to be transfected
Expi293 PRO Transfection Reagent	4.5 µL per mL of culture volume to be transfected (0.45%)
Expi293 PRO Feed	80 µL per mL of culture volume transfected (8%)
Expi293 PRO Enhancer	6 µL per mL of culture volume transfected (0.6%)

Results

As noted, before transfection studies were conducted, cells were grown to peak density in each vessel format to verify that Expi293 PRO cells exhibited normal growth characteristics and remained healthy under each set of culture conditions compared to 125 mL shake flask controls. After testing a range of culture volumes and shake speeds to pinpoint the most favorable growth conditions, we applied these optimized parameters to begin our transfection work. For protein expression analysis, cultures were transfected with a human IgG monoclonal antibody construct and incubated for 7 days. For each format tested, 125 mL shake flasks were transfected in parallel as controls. Antibody titers were determined using Octet™ ProA biosensors on a ForteBio™ Octet™ Blue system. Results are described by vessel format.

Extra-high 24 deep-well plates

Orbital diameter	Recommended speed
25 mm	275 rpm
50 mm	230 rpm

Extra-high 24 deep-well plates were evaluated using transfection volumes of 5 mL and 6 mL on 25 mm and 50 mm orbital shakers (Figure 1). Optimal performance was achieved at 275 rpm for the 25 mm orbital shaker and 230 rpm for the 50 mm orbital shaker.

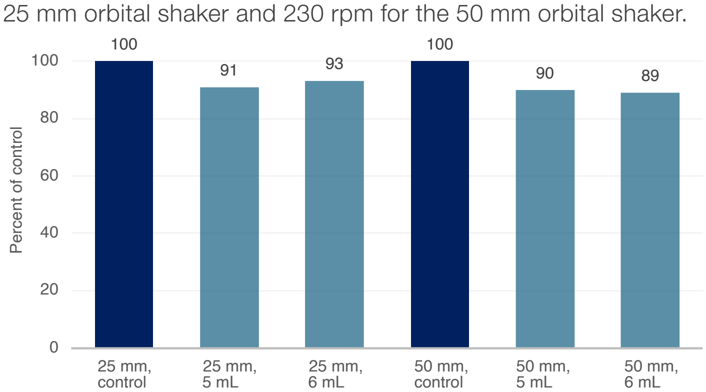

Figure 1. Performance of the Expi293 PRO Expression System with extra-high 24 deep-well plates. Titers are shown as percent of the 125 mL shake flask control. Volumes shown refer to the culture volumes at the time of transfection.

Caution: When placed in the clamping system, these plates may become top-heavy, which at higher shaking speeds can compromise adhesion to the shaker's sticky pads and potentially lead to clamp detachment.

The clamps adhere to sticky pads or tape on the shaker platform. Make sure there is proper adhesion to ensure that the clamping system does not detach from the platform during shaking. It is also best to fill every well of the plate to weigh the plate down equally and reduce the likelihood of the clamp detaching from the sticky surface.

6 deep-well plates

Orbital diameter	Recommended speed
25 mm	225 rpm
50 mm	180 rpm

6 deep-well plates were evaluated using transfection volumes of 20–40 mL on both 25 mm and 50 mm orbital shakers (Figure 2). Optimal performance was achieved at 225 rpm for the 25 mm orbital shaker and 180 rpm for the 50 mm orbital shaker.

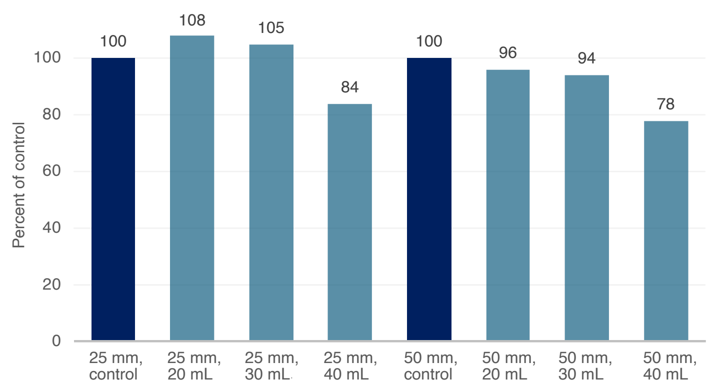

Figure 2. Performance of the Expi293 PRO Expression System with 6 deep-well plates. Volumes shown refer to the culture volumes at the time of transfection.

Caution: At higher transfection volumes (i.e., 40 mL), culture medium may splash onto the sandwich cover when shaking. This may prevent proper gas exchange and consequently reduce protein titer, as seen in the 40 mL transfection volume data in Figure 2.

The clamps adhere to sticky pads or tape on the shaker platform. Make sure there is proper adhesion to ensure that the clamping system does not detach from the platform during shaking. It is also best to fill every well of the plate to weigh the plate down equally and reduce the likelihood of the clamp detaching from the sticky surface.

Nunc 50 mL Bioreactor Tubes

Orbital diameter	Recommended speed
25 mm	225 rpm
50 mm	180 rpm

Nunc 50 mL Bioreactor Tubes were evaluated using transfection volumes of 10–25 mL in both vertical (90°) and angled (67.5° from horizontal) orientations on 25 mm and 50 mm orbital shakers (Figure 3). Optimal performance was achieved for both vertical and angled 50 mL bioreactor tubes at 225 rpm for the 25 mm orbital shaker and 180 rpm for the 50 mm orbital shaker. Note that the angled orientation allows for expression at higher volumes without loss compared to 125 mL shake flask controls.

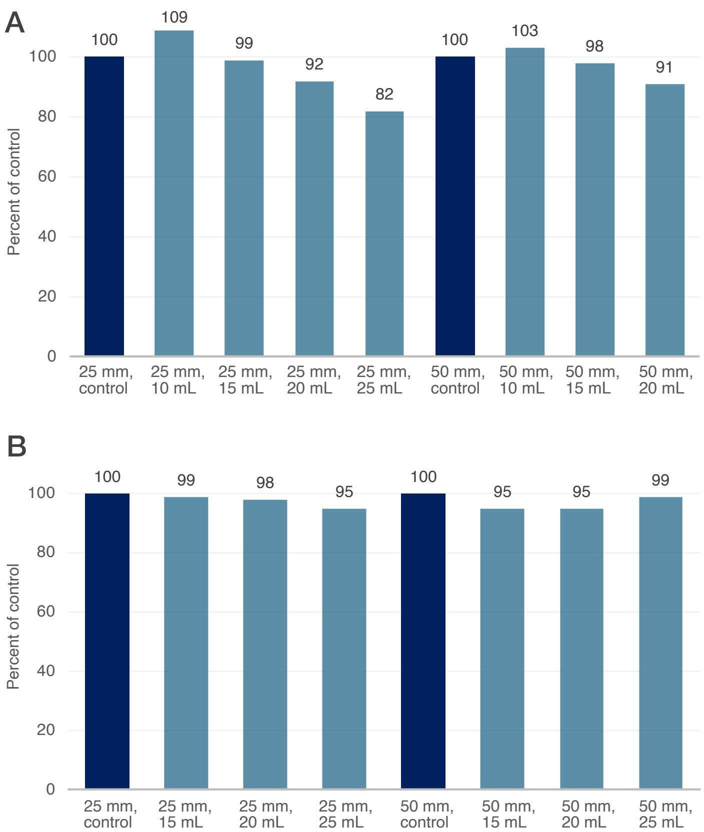

Figure 3. Performance of the Expi293 PRO Expression System with Nunc 50 mL Bioreactor Tubes. Shown are results of tubes tested in **(A)** vertical and **(B)** angled orientations. Volumes shown refer to the culture volumes at the time of transfection.

Caution: Achieving the proper angle in this orientation is critical. When the tubes are positioned too close to horizontal, cultures may contact the filter cap during shaking, resulting in excessive foaming, impaired gas exchange, and poor performance.

600 mL TubeSpin Bioreactor Tubes

Orbital diameter	Recommended speed
25 mm	225 rpm
50 mm	180 rpm

The 600 mL TubeSpin Bioreactor Tubes were evaluated in a vertical orientation using transfection volumes of 250–400 mL on 25 mm and 50 mm orbital shakers (Figure 4). Optimal performance was achieved at 225 rpm for the 25 mm orbital shaker and 180 rpm for the 50 mm orbital shaker. For this application, a specialized shaker platform and tube racks are required to hold the tubes while working in a biosafety cabinet. See Table 2 for ordering information.

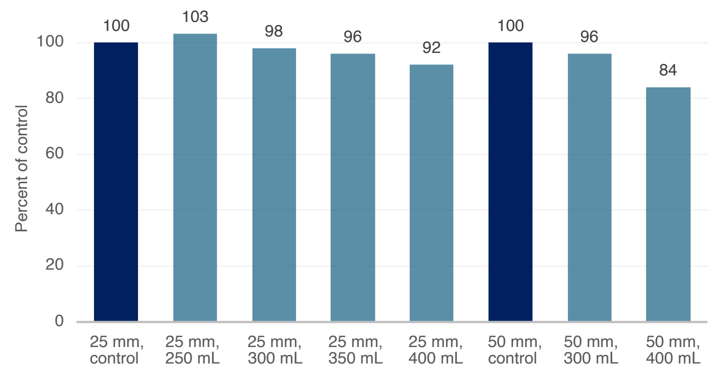

Figure 4. Performance of the Expi293 PRO Expression System with TubeSpin 600 mL Bioreactor Tubes. Volumes shown refer to the culture volumes at the time of transfection.

Caution: Due to the large working volumes accommodated by this format and the conical bottom shape, careful handling is required during all manipulations. Tubes should be kept in a vertical orientation throughout all manipulations to prevent the culture from touching the filter cap.

7 L shake flasks

Orbital diameter	Recommended speed
25 mm	145 rpm
50 mm	110 rpm

Given the large culture volume required by this format, we chose to determine optimal conditions for 7 L shake flasks by first culturing cells at 0.3 x 10^6 cells/mL at 3 L, 4 L, or 5 L culture volumes and determining viable cell density and percent viability 4 days post-inoculation. Flasks cultured at 3 L or 4 L conditions showed cell growth comparable to 125 mL shake flask controls. Cell growth at the 5 L culture condition lagged slightly but still reached the recommended density of 4–6 x 10^6 cells per mL of culture to proceed with transfection.

Cells were then seeded at 1.6 x 10^6 cells/mL for a day 2 subculture prior to transfection at 3, 4, or 5 L volumes. Once again, slightly lower cell densities were obtained with the 5 L cultures; however, the cultures were still within the acceptable density range of 6–8 x 10^6 viable cells/mL to proceed with transfection.

Cells were transfected at 3, 4, or 5 L culture volumes with optimal performance achieved at 145 rpm for the 25 mm orbital shaker and at 110 rpm for the 50 mm orbital shaker (Figure 5).

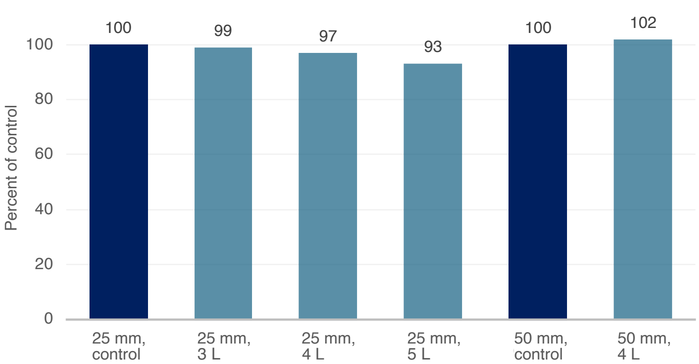

Figure 5. Performance of the Expi293 PRO Expression System with 7 L shake flasks. Volumes shown refer to the culture volumes at the time of transfection.

Caution: Due to the large working volumes accommodated by this format, careful handling is required particularly when moving the flasks in and out of the biosafety cabinet to prevent the culture from touching the filter cap.

Conclusions

The Expi293 PRO Expression System has been demonstrated to have excellent scalability from milliliter to multi-liter scales. Here we further adapted the expression protocols for the Expi293 PRO Expression System to allow for additional flexibility using vessel formats that are becoming increasingly prevalent in the protein expression community. Our results confirm that the Expi293 PRO Expression System is well suited for protein expression using various larger-volume, multi-well plate formats, conical-bottom shake flasks, and large-scale shake flasks allowing for upwards of 5 L of expression volume. For recommended shake speeds for each format tested, see Table 4.

Although a broad range of conditions were tested in this study, in certain instances additional optimization may be required to account for alternative transfection volumes, shake speeds, or orbital diameters. In general, higher transfection volumes will require higher shaking speeds; however, caution must be taken to prevent wetting of the gas exchange filters by splashing of higher volume cultures. Optimal shaking speeds for 19 mm and 25 mm shakers are typically similar to one another (±5 rpm) across different vessel formats; however, when moving from 19 mm or 25 mm shakers to 50 mm shakers, significantly different vessel geometries make it impractical to assign a consistent conversion factor for shaking speeds. Thus, it is best to determine optimal shaking speeds empirically for each vessel to help ensure maximal performance.

Table 4. Summary of recommended shake speeds tested for each vessel.

Type of vessel	Orbital diameter	Speed
Extra-high 24 deep-well plates	25 mm	275 rpm
	50 mm	230 rpm
6 deep-well plates	25 mm	225 rpm
	50 mm	180 rpm
Nunc 50 mL Bioreactor Tubes (vertical and angled orientations)	25 mm	225 rpm
	50 mm	180 rpm
TubeSpin 600 mL Bioreactor Tubes	25 mm	225 rpm
	50 mm	180 rpm
7 L shake flasks	25 mm	145 rpm
	50 mm	110 rpm

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