Protein expression

Nunc bioreactor tubes for various protein expression systems

Introduction

Recombinant protein expression is widely used in applications from investigating biological functions to large-scale production of enzymes, antibodies, and vaccines. To investigate and identify such proteins, researchers require high-throughput screening of proteins prior to scale-up.

For this purpose, the most frequently adopted expression systems are mammalian, bacterial, and insect cell expression systems. Along with the appropriate expression system, choosing the right bioreactor is also important for successful screening and production of the recombinant proteins. High-throughput screening often requires small-scale systems, but the small-scale systems need to be as efficient as large vessels in terms of cell growth and protein production.

We introduce the Thermo Scientific™ Nunc™ 50 mL Bioreactor Tube for high-throughput screening of critical proteins prior to scale-up production. Here we demonstrate the use of Nunc bioreactor tubes to support growth and protein expression in mammalian, bacterial, and insect expression systems, in comparison to an alternate supplier's product and the Thermo Scientific™ Nalgene™ 125 mL Single-Use PETG Erlenmeyer Flasks with Plain Bottom (Nalgene shake flask).

Materials

- Nunc 50 mL Bioreactor Tube (Cat. No. 332260)
- 50 mL bioreactor tube from another supplier (supplier A)
- Nalgene 125 mL Single-Use PETG Erlenmeyer Flasks with Plain Bottom (Cat. No. 4115-0125)
- Gibco™ Expi293™ Expression System Kit (Cat. No. A14635)
- Gibco™ ExpiCHO™ Expression System Kit (Cat. No. A29133)

- Invitrogen™ BL21-AI™ One Shot™ Chemically Competent *E. coli* (Cat. No. C607003)
- Gibco™ ExpiSf™ Expression System Starter Kit (Cat. No. A38841)
- Eppendorf™ New Brunswick™ Innova™ 44 Stackable Incubator Shaker, stackable up to 3-fold, 230 V/50 Hz, orbit 2.5 cm (1 in.) (Eppendorf Cat. No. M1282-0002)

Methods

To demonstrate the efficiency of Nunc bioreactor tubes, we compared growth and protein expression in them against supplier A's bioreactor tube and a Nalgene 125 mL Erlenmeyer shake flask. Each of the expression systems' recommended protocols were followed as below.

Mammalian expression

To check mammalian expression, we used Expi293F and ExpiCHO-S cells:

- Protocol and more information for the Expi293 Expression System
- Protocol and more information for the ExpiCHO Expression System

Bacterial expression

To check bacterial expression, we used BL21-AI One Shot Chemically Competent *E. coli* cells:

- Protocol for BL21-AI One Shot Chemically Competent *E. coli*

Insect expression

To check insect cell expression, we used ExpiSf9 cells:

- Protocol and more information for the ExpiSf Expression System Starter Kit

Briefly, the required numbers of cells were seeded in the bioreactor tubes and flasks, then incubated to grow until the desired cell density was reached. The cells were then transfected with the desired gene as per the protocol, and growth kinetics of each expression system were measured daily. Samples were harvested on the recommended day and protein titers were measured.

Bioreactor tube handling:

1. Remove the Nunc 50 mL Bioreactor Tube from the packaging in a sterile environment.
2. The Nunc bioreactor tubes were designed with an adjustable 4-level vented cap with a 0.2 µm filter membrane for controlled gas exchange.
3. Ensure the airflow dial on the screw cap is in the open position with all vents exposed (Figure 1).
4. Twist off the screw cap of the bioreactor tube and fill it with the medium and inoculum according to the manufacturer's protocol.
5. Place the screw cap back on the bioreactor tube and twist it on tightly to avoid leakage. Gas exchange occurs through the membrane fixed on the cap.
6. If the membrane gets wet during the experiment, the screw cap must be replaced to avoid inefficient gas exchange.

Figure 1. The airflow dial on the screw cap of the Nunc 50 mL Bioreactor Tube in the open position.

Results

Mammalian expression system

ExpiCHO-S and Expi293F cells were seeded in a Nunc bioreactor tube, supplier A's bioreactor tube, and a Nalgene shake flask. The cells were grown for 5 days and their growth kinetics were compared.

On each day, viable cell density was measured to monitor cell growth and cell viability for up to 5 days. ExpiCHO cells cultured in the Nunc and supplier A's bioreactor tubes produced comparable maximum cell densities, with an average of 12.1 x 10^6 cells/mL on day 5, whereas the Nalgene shake flask produced a cell density of 14.6 x 10^6 cells/mL (Figure 2A). Expi293F cells grown in the Nunc and supplier A's bioreactor tubes yielded averages close to 4 x 10^6 cells/mL, while the Nalgene shake flask yielded 7.8 x 10^6 cells/mL (Figure 2B). Meanwhile, the mid-log phase cell viability was greater than 95% for both ExpiCHO and Expi293 cells in all vessels. Based on these data, there is no significant difference in the growth kinetics and viability of ExpiCHO and Expi293F cells grown in the two brands of bioreactor tubes.

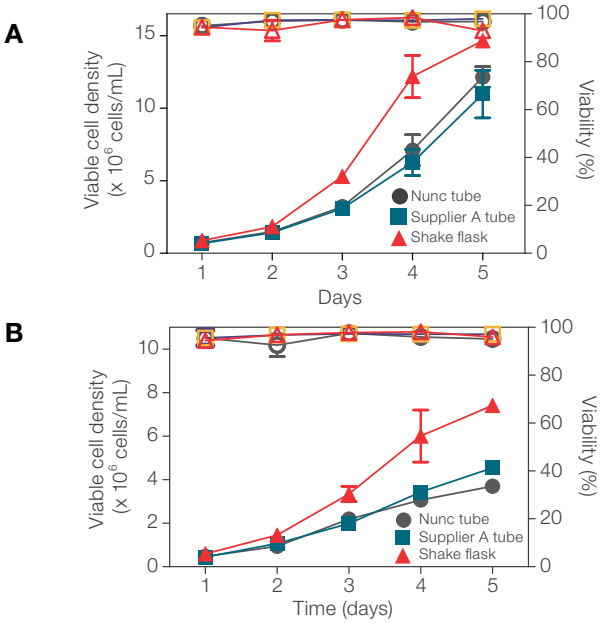

Figure 2. Growth comparison of ExpiCHO-S and Expi293F cells in a Nunc bioreactor tube, supplier A's bioreactor tube, and a Nalgene shake flask. (A) ExpiCHO-S cells seeded at a density of 0.3 x 10^6 cells/mL were grown for 5 days. Daily measurements of viable cell density and cell viability are represented. On day 5, ExpiCHO-S cells produced equivalent cell densities with no significant difference between the two brands of bioreactor tubes. However, the shake flask produced a higher cell density than both bioreactor tubes. **(B)** Expi293F cells seeded at a density of 0.5 x 10^6 cells/mL were grown for 5 days. Daily measurements of viable cell density and cell viability are shown. For both growth attributes, the data demonstrate no significant difference between the Nunc bioreactor tube and supplier A's bioreactor tube. However, on day 5, the shake flask produced a higher cell density than the bioreactor tubes, consistent with ExpiCHO-S cell growth in the various vessels (n = 2 passages; error bars represent mean ± SEM).

To check protein expression in mammalian cells, ExpiCHO-S and Expi293F cells were transfected with a human IgG plasmid using standard protocols from the ExpiCHO Expression System Kit and the Expi293 Expression System Kit, respectively. ExpiCHO-S cells were harvested on day 4 and day 7 and protein titer was estimated; Expi293F cells were harvested on day 3 and day 6 and protein titer was estimated. Results for the ExpiCHO-S cells show that the Nunc and supplier A's bioreactor tubes produced equivalent expression of IgG, as measured by protein titer, whereas the Nalgene shake flask produced a slightly higher titer (Figure 3A). Results for the Expi293F cells show that the Nunc bioreactor tube produced IgG titers equivalent to those of supplier A's bioreactor tube and the Nalgene shake flask (Figure 3B). Thus, the Nunc bioreactor tube is nearly as efficient as the shake flask for protein production.

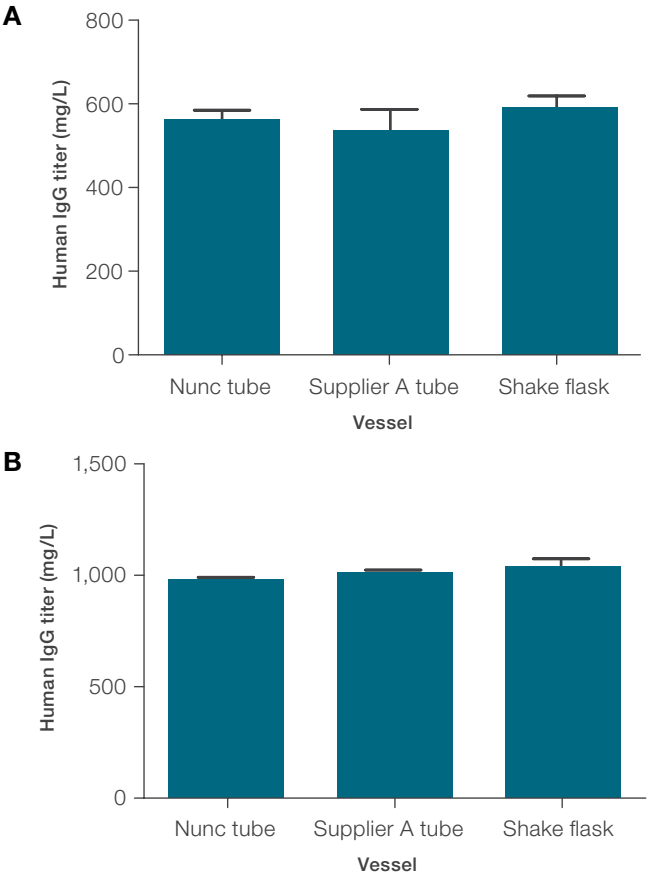

Figure 3. Comparison of expressed protein yields in the Nunc bioreactor tube, supplier A's bioreactor tube, and the Nalgene shake flask. (A) Human IgG was expressed from a plasmid with the ExpiCHO Expression System and harvested on day 7. Respective protein titers are represented. **(B)** Human IgG was expressed from a plasmid with the Expi293 Expression System and harvested on day 7. Respective protein titers are represented. The data show no significant difference in protein expression levels between the Nunc and supplier A's bioreactor tubes, and titers nearly comparable to those of the Nalgene shake flask (n = 2; error bars represent mean ± SEM).

Insect expression system

To assess insect cell growth, ExpiSf9 cells were seeded in a Nunc bioreactor tube, supplier A's bioreactor tube, and a Nalgene shake flask. Viable cell density and cell viability were measured daily for 8 days. The growth kinetics data showed no significant difference in cell density and viability between the Nunc bioreactor tube and Nalgene shake flask. To evaluate protein expression, ExpiSf9 cells were transfected with green fluorescent protein (GFP)-tagged plasmid and grown for 8 days. Cells were harvested and their relative GFP expression levels were compared. The Nunc bioreactor tube, supplier A's bioreactor tube, and the Nalgene shake flask produced equivalent expression with no significant differences (Figure 4).

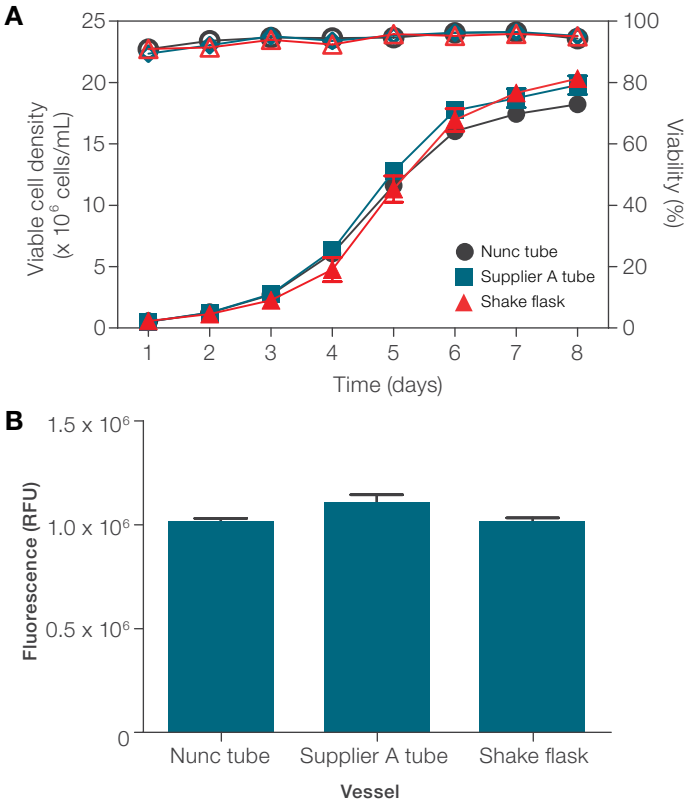

Figure 4. Comparison of yields from the ExpiSf Expression System in the Nunc bioreactor tube, supplier A's bioreactor tube, and the Nalgene shake flask. ExpiSf9 cells seeded at a density of 0.3 x 10^5 cells/mL were grown for 8 days in the three vessel types. **(A)** Cell density and viability were assessed on each day and are represented. Growth results were comparable for all conditions. **(B)** GFP expression data collected after 8 days of transfection are represented. Data demonstrate no significant difference in expression among the three vessel types (n = 2; error bars represent mean ± SEM).

Bacterial expression system

BL21-AI One Shot Chemically Competent *E. coli* cells were transformed with three genes of interest (*EIF4B*, *CDKL5*, *TDR3*). The cells were then subcultured at a ratio of 1:50 (e.g., 1 mL of culture per 50 mL of medium) into a Nunc bioreactor tube, supplier A's bioreactor tube, and a Nalgene shake flask. Samples were taken every hour to measure the optical density (OD) of the culture for up to 14 hours. The growth kinetics in the Nunc bioreactor tube were equivalent to those in supplier A's bioreactor tube, and comparable to those in the Nalgene shake flask, with no significant differences (Figure 5).

To assess protein expression, cells grown from the three conditions were evaluated with urea-PAGE. Cells were harvested, lysed with urea, and centrifuged. The supernatants and pellets were collected and analyzed by electrophoresis. Results showed that transformed bacterial cells actively expressed all three proteins at 37°C in the Nunc and supplier A's bioreactor tubes, without significant differences between the expression levels in the two tube types (Figure 6).

Conclusion

We demonstrated that the Nunc 50 mL Bioreactor Tube has comparable performance in growth and protein expression in various expression systems compared to another supplier's bioreactor tube. In addition, we have shown that the Nunc bioreactor tube produces results equivalent to those of the 125 mL Nalgene shake flask. Accordingly, the Nunc 50 mL Bioreactor Tube enables high-throughput screening of proteins at a competitive cost and can be used in process optimization of various protein expression systems.

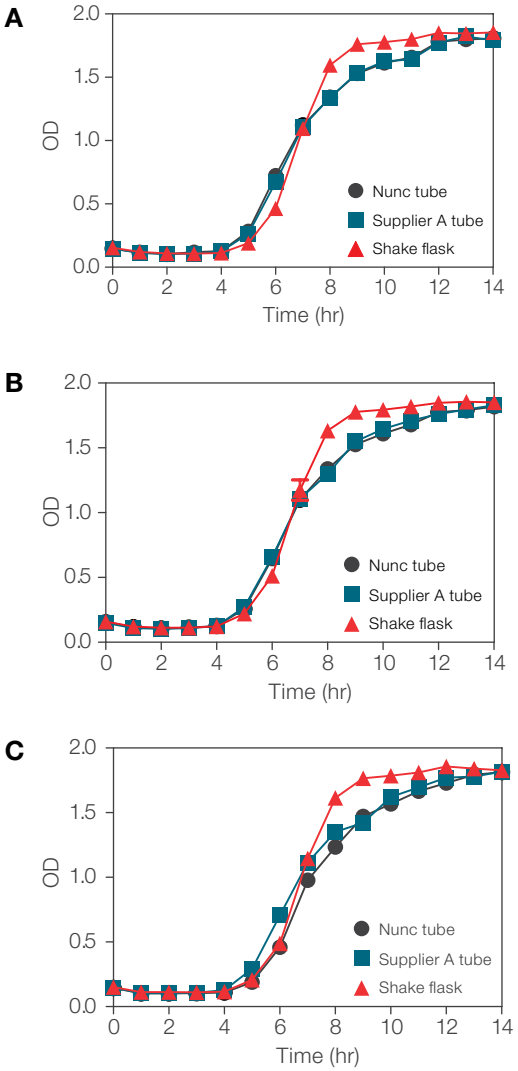

Figure 5. Comparison of protein expression in BL21-AI chemically competent *E. coli* in the Nunc bioreactor tube, supplier A's bioreactor tube, and the Nalgene shake flask. Growth kinetics of cells transformed with plasmids carrying **(A)** *EIF4B*, **(B)** *CDKL5*, and **(C)** *TDRD3* were monitored every hour, and respective data are represented. Comparable optical densities were achieved with all three plasmids tested, with no significant differences among the vessel types (n = 2, error bar represents mean ± SEM).

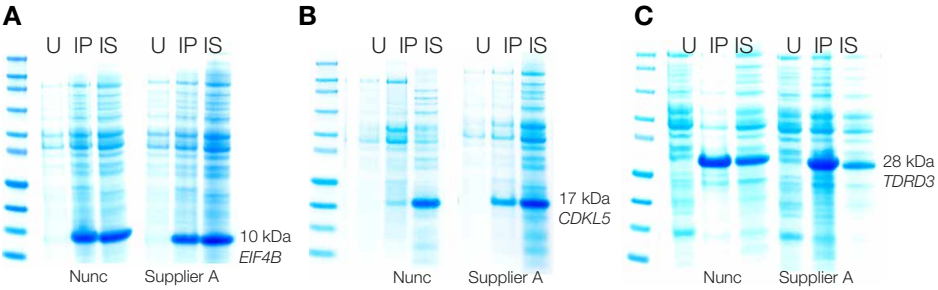

Figure 6. Comparison of protein expression in Nunc and supplier A's bioreactor tubes. BL21-AI One Shot *E. coli* cells were transformed with plasmids carrying **(A)** *EIF4B*, **(B)** *CDKL5*, and **(C)** *TDRD3*. Urea-PAGE analysis shows all three transformations produced equivalent expression in both Nunc and supplier A's 50 mL bioreactor tubes. A protein band of the expected molecular mass was seen in all conditions. U: untransformed cells, IP: pellet from transformed and induced cells, IS: supernatant from transformed and induced cells.

Ordering information

Product	Quantity	Cat. No.
Nunc 50 mL Bioreactor Tube	Case of 90	332260
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom	Case of 24	4115-0125
Expi293 Expression System Kit	1 kit	A14635
ExpiCHO Expression System Kit	1 kit	A29133
BL21-AI One Shot Chemically Competent *E. coli*	20 x 50 µL/tube	C607003
ExpiSf Expression System Starter Kit	1 kit	A38841
Eppendorf New Brunswick Innova 44 Stackable Incubator Shaker, stackable up to 3-fold, 230 V/50 Hz, orbit 2.5 cm (1 in.)	1 device	M1282-0002 (Eppendorf)

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