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Scalable Results 30mL-2L in Optimum Growth® Flasks with ExpiCHO™ Expression System after Transfection

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Abstract

The Thomson Optimum Growth® line of flasks provides robust growth and protein expression of CHO suspension cells and their derivatives. One of the most popular lines is the ExpiCHO™ Expression System from Thermo Fisher Scientific. Although a proven workhorse for mABs and antigen expression, difficulty in scaling up beyond 500mL has been a limitation of the system. Below we present a method for scaling up to 2L, thereby improving efficiency in the workflow and economy of space.

Introduction

The Thomson Optimum Growth® Flask is designed to enhance cell culture processes, particularly for suspension cell lines such as ExpiCHO-S™. This scientific application note details an improved transfection protocol utilizing the Thomson Optimum Growth® Flask to improve cell viability and protein yield during recombinant protein production. The flask's design promotes effective gas exchange and uniform mixing, which are critical for optimal cell growth and transfection efficiency.

Equipment

1. **Cell Culture Equipment:**
   A. Thomson Optimum Growth® Flask (5L) (Cat. No. 931116)
   B. INFORS HT Multitron incubator shaker
2. **Reagents:**
   A. ExpiCHO-S™ cells
   B. OptiPRO™ SFM
   C. ExpiFectamine™ CHO reagent
   D. Plasmid DNA (target protein)
3. **Consumables:**
   A. 250mL sterile centrifuge bottles
   B. Pipettes and pipette tips
   C. Centrifuge tubes
4. **Analytical Equipment:**
   A. Viable cell count analyzer
   B. Protein quantification assays (e.g., BCA assay)

Improved Protocol

Day -1 | Cell Preparation

1. **Cell Splitting:**
   A. Split ExpiCHO-S™ seed cells to achieve a final density of 4 x 10^6 viable cells/mL.
   B. Incubate the cells overnight at 37°C with 8% CO_2, on a platform shaker with a 2" orbit set to 100 rpm. Shakers with a 1" orbit should be set to 140 rpm.A speed adjustment post-transfection will reduce the speed on a 2" orbital shaker to 80rpm, or on a 1" orbital shaker to 100rpm. (Refer to Table 2)
   C. It is crucial to maintain humidity levels between 50-55% for optimal experimental conditions. If humidity control is not available, an alternative method is to use Thomson 1.6L flask filled with water, leaving the cap off to help maintain required moisture levels.

Day 0 | Transfection

1. **Cell Setup:**
   A. Prepare 1500mL of ExpiCHO-S™ cells at a concentration of 6 x 10^6 viable cells/mL in the 5L Thomson Optimum Growth® Flask.
   B. Set the shaker to maintain a temperature of 37°C and 8% CO_2.
2. **Cell Acclimatization:**
   A. Allow the cells to shake for 30 minutes to 1 hour to acclimate to the new density, enhancing the conditions for successful transfection.
3. **DNA Calculation:**
   A. Calculate the required amount of DNA for transfection (1µg total plasmid DNA per mL of culture), totaling 1.5 mg for the 1500 mL culture. (Refer to Table 1 for working volumes for different size Thomson flasks)
4. **Material Preparation:**
   A. Assemble all materials in the hood for transfection preparation, including:
   B. Cells slated for transfection
   C. OptiPRO™ SFM
   D. ExpiFectamine™ CHO reagent
   E. Plasmids for transfection
5. **DNA Transfection Solution Preparation:**
   A. Add 60mL of cold OptiPRO™ to a sterile centrifuge bottle and incorporate the calculated DNA amount. Label this bottle as "DNA".
6. **ExpiFectamine™ CHO Reagent Preparation:**
   A. Add 56 mL of cold OptiPRO™ to a separate sterile centrifuge bottle, then add 4.8mL of ExpiFectamine™ CHO reagent. Label as "ExpiFectamine™".
7. **Complex Formation:**
   A. Combine the contents of the "ExpiFectamine™" bottle with the "DNA" bottle within 5 minutes to optimize complex formation.
8. **Mixing:**
   A. Invert the mixture 5 times to ensure thorough mixing, allowing it to sit for 30 seconds to 1 minute for stabilization.
9. **Complex Addition:**
   A. Add the ExpiFectamine™ DNA complexes to the cells in the flask, gently swirling to ensure uniform distribution.
10. **Shaking Adjustment:**
   A. Transfer the flask back to the shaker, **reducing the speed to 80RPM** while maintaining 37°C with 8% CO_2. Keep reduced rpm for the remainder of the expression. For a 1" orbital shaker reduce speed to 110rpm for the remainder of the expression.

Day 1 | Post-Transfection

1. **Enhancement and Feeding:**
   A. Feed the cells with the calculated amounts based on Thermo Fisher Scientific

guidelines for optimal titer (e.g., Higher Titer, Max Titer).

2. **Temperature Adjustment:**
   A. After 24 hours, reduce the temperature to 32°C while maintaining 8% CO_2 for the remainder of the expression phase to enhance protein production.

Pre-Transfection		Post-Transfection	
Orbit	RPM	Orbit	RPM
2"	100	2"	80
1"	140	1"	110

Table 2: Shaker Speed

ExpiCHO Transfections

Expression Size	50mL	100mL	200mL	500mL	1L	2L
Thomson Flask Size	125mL (Cat. No. 931110)	250mL (Cat. No. 931111)	500mL (Cat. No. 931112)	1.6L (Cat. No. 931113)	2.8L (Cat. No. 931114)	5L (Cat. No. 931116)
Volume at 6E6 cells /mL	37.5mL	75mL	150mL	375mL	750mL	1500mL
Opti-MEM™ for ExpiFectamine™	1.4mL	2.8mL	5.6mL	14mL	28mL	56mL
ExpiFectamine™	0.12mL	0.24mL	0.48mL	1.2mL	2.4mL	4.8mL
Opti-MEM™ for DNA	1.5mL	3mL	6mL	15mL	30mL	60mL
DNA	0.0375/ (DNA Concentration)	0.075/ (DNA Concentration)	0.15/ (DNA Concentraiton)	0.375/ (DNA Concentration)	0.75/ (DNA Concentration)	1.5/ (DNA Concentration)
Enhancer	0.225	0.45mL	0.90mL	2.25mL	4.5mL	9mL
Feed Day 1	9mL	18mL	36mL	90mL	180mL	360mL
Feed Day 5	6mL	12mL	24mL	60mL	120mL	240mL

Table 1: Working volumes for different size Thomson flasks

Key Notes

Incubation and Transfection: Shortening the incubation time with the transfection reagent and adjusting shaking speed posttransfection enhances cell viability.

Temperature Management: Dropping the temperature after enhancing and feeding cells is recommended, and maintaining low shaking speeds post-transfection is beneficial.

Scale Considerations: For cultures under 1L, modify shaking speed and temperature as opposed to complexation time, as smaller flasks operate more efficiently.

Humidity Control: Controlling humidity is essential to prevent significant volume loss due to evaporation, which can negatively impact cell health. Recommend using a 1.6L flask filled with 1L of purified water inside incubator to help prevent loss to evaporation.

Result

Implementing the improved transfection protocol in the Thomson Optimum Growth® Flask resulted in a notable increase in protein production. The cell viability results obtained from this experimental setup were found to be comparable to those achieved using standard expression methods, indicating effective transfection and cell maintenance. In addition, this method will reduce the number of flasks used and space needed. The INFORS HT Multitron incubator shaker used in this experiment helped ensure precise control and reliability throughout the scale-up of CHO suspension cultures. Its adjustable orbital throw, flexible parameter settings, and robust capacity enhance transfection efficiency, cell viability, and protein yield, making it ideal for advancing recombinant protein production. The integration of Thomson Optimum Growth® Flasks maximizes protein production in ExpiCHO™ expression system, overcoming the volume limitations seen in competitor flasks.

Conclusion

The Thomson Optimum Growth® Flasks proves to be a valuable tool in bioprocessing, provides a solution to the volume constraints of competitor flasks and offering researchers a dependable solution for optimizing protein production.

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