Large Scale Transient Transfection for Expression of Recombinant Protein in Corning® 5L Erlenmeyer Flasks

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Protocol

Introduction

As a product of biopharmaceuticals, recombinant protein plays a significant role in medicine. Using genetic engineering technology, cells or animals themselves can be turned into "factories" for mass production of drugs. In the current biopharmaceutical field, mammalian cell expression systems have become the mainstream protein-based technology for producing recombinant proteins for therapeutic use. Mammalian cells produce high levels of recombinant proteins, and their physical and chemical properties, as well as biological functions are similar to those of natural proteins.

Transient transfection is one of the methods of introducing DNA into eukaryotic cells. During transient transfection, recombinant DNA is introduced into mammalian cells to obtain temporary but high-level expression of the target gene[1]. Transient transfection is widely used because of its advantages, such as a simple and rapid procedure, high expression efficiency, and high safety, and because there is no need for gene screening[2].

Corning 5L Erlenmeyer flasks were selected to expand suspension cell culture because the PETG (polyethylene terephthalateco-1,4-cylclohexylenedimethylene terephthalate) material does not produce a 3,5-dinitro-bisphenol A leachate, which inhibits cell growth. In addition, the high liquid surface area to volume ratio results in better culture aeration and mixing.

Materials

- ExpiCHO-S™ cells (Thermo Fisher A29127)
- ExpiCHO™ Expression medium (Thermo Fisher A2910001)
- ExpiCHO Expression System kit (Thermo Fisher A29133)
- Axygen® Plasmid Purification kits (Corning AP-MX-P-25)
- Corning 125 mL Erlenmeyer flasks (Corning 431143)
- Corning 250 mL Erlenmeyer flasks (Corning 431144)
- Corning 500 mL Erlenmeyer flasks (Corning 431145)
- Corning 1L Erlenmeyer flasks (Corning 431147)
- Corning 5L Erlenmeyer flasks (Corning 431685)
- Axygen 1.5 mL microcentrifuge tube (Corning MCT-150-C-S)
- Corning 15 mL centrifuge tube (Corning 430053)
- Corning 50 mL centrifuge tube (Corning 430290)
- Axygen 1 mL pipet tip (Corning TF-1000-R-S)
- Corning 5 mL pipet (Corning 4487)

Procedure

Plasmids preparation

1. Use Axygen plasmid purification kit to prepare the high purified plasmid. The preparation follows the product's standard protocol. The 260/280 ratio of the plasmid solution is ~1.8. The 260/230 ratio is 2.0 to 2.2, and the endotoxin level is less than 5 EU/mg. To ensure the sterility of plasmid, you can consider filtrating the plasmid through a 0.22 µm filter.

Cell preparation

2. Seed ExpiCHO-S cells in 30 ml pre-warmed ExpiCHO Expression Medium, using a Corning 125 mL Erlenmeyer Flask. Incubate at 37°C with 8% CO_2 on a shaker platform. Set the shake speed to 110 ± 5 rpm for a shaker with a 50 mm orbital diameter. Monitor the cell density and viability till the cell density reaches 4 x 10^6 to 6 x 10^6 viable cells/mL.

NOTE: To maintain in the logarithmic growth phase, the cells are subcultured when cell density reached 4 x 10^6 to 6 x 10^6 viable cells/mL in 3 to 4 days culturing. The seeding density is 0.2 to 0.4 x 10^6 viable cells/mL Maintain cell viability >95% in culture. When the cell density is too high, cells tend to aggregate and turn to cell clumps. Do not pipet cells. Transfer the cell suspension for passaging.

3. Transfer the calculated volume of cells suspension to a Corning® 250 mL Erlenmeyer flask. Add fresh, pre-warmed ExpiCHO Expression Medium to the flask and make the total volume to 60 mL. Keep the initial cell density consistent with the seeding density in Step 2. Incubate the cell suspension with the same conditions in Step 2 until the cell density reaches 4 x 10^6 to 6 x 10^6 viable cells/mL.
4. Expand cells in 500 mL, 1L, and 5L Corning Erlenmeyer flasks, sequentially. Transfer the calculated volume of cells suspension to a larger Corning Erlenmeyer flask. Add fresh, pre-warmed ExpiCHO™ Expression Medium to the flask. The total volumes follow the initial volume recommended in the table of Shaker Bottle Specifications. Keep the initial cell density in consistent with the seeding density in Step 2. Incubate the cell suspension with the same condition in Step 2 until the cell density reaches 4 x 10^6 to 6 x 10^6 viable cells/mL.

Transfection steps

5. The day before transfection, cells are seeded in to a 5L Corning Erlenmeyer flask at 3 x 10^6 to 4 x 10^6 viable cells/mL. Let cells grow overnight to reach the desired density (7 x 10^6 to 10 x 10^6 viable cells/mL, viability >95%).

NOTE: If the cell density is too high, dilute with fresh medium, discard excess cells, and do not continue subculture.

6. Dilute the plasmid with 40 mL to 50 mL OptiPRO medium (included in the ExpiCHO Expression System kit), using a 125 mL or 250 mL Corning Erlenmeyer flask. 0.8 to 1.2 mg DNA per liter of culture volume is diluted in this step. Mix the tube by inverting gently.
7. Dilute the ExpiFectamine™ CHO reagent with 40 mL to 50 mL OptiPRO medium, using a 50 mL centrifuge tube. 0.8 to 1 mL per liter of culture volume is diluted in this step. Mix the tube by inverting gently.
8. Pour the diluted ExpiFectamine CHO reagent into the diluted plasmid at 1:1 volume ratio. Mix by inverting gently, then stand the mixture for 1 to 5 min. at room temperature.

NOTE: ExpiFectamine CHO reagent should be added to DNA within 30 second to 5 minutes after dilution to avoid a decrease of protein expression.

9. Gently pour the mixture prepared in Step 8 into the 5L Corning Erlenmeyer flask from Step 5. Gently swirl the flask during addition.
10. Put the flask back to the shaker. Incubate the cells at 37°C with 8% CO_2. Reduce the rotational speed of the shaker to 90 to 100 rpm. It is Day 0.
11. Eighteen to twenty-two hours after transfection, add ExpiFectamine CHO Enhancer (included in the ExpiCHO Expression System kit) to the cell suspension, 6 mL per liter of culture medium. And add ExpiCHO Feed (included in the ExpiCHO Expression System kit) to the cells suspension, 160 mL per liter of culture medium. Transfer the flask to a 32°C incubator with 5% CO_2, and shake the flask as in Step 10.
12. On Day 5, add the second volume of ExpiCHO Feed to the cell suspension, 160 mL per liter of culture medium. Immediately return the flask to 32°C incubator and continue shaking.
13. Harvest cells in 8 to 14 days after transfection, keep the viability >75%.

The following table summarizes the transfection specifications when using a different size of Erlenmeyer flasks.

Shaker Bottle Specifications

Flask Size	125 mL	250 mL	500 mL	1L	5L
Initial volume	30 mL	60 mL	100 to 200 mL	100 to 300 mL	1 to 2.5L
Rotational speed		110 to 120 rpm after transient 90 to 100 rpm			
Final culture (approx.)	40 mL	84 mL	140 to 280 mL	196 to 420 mL	1.4 to 3.5L

References

1. Jain NK, et al. A high-density CHO-S transient transfection system: Comparison of ExpiCHO and Expi293. Protein Expression and Purification, 134:3846. 2017.
2. Rosser MP, et al. Transient transfection of CHO-K1-S using serum-free medium in suspension: A rapid mammalian protein expression system. Protein Expression and Purification, 40:237-243, 2005.

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