ExpiCHO Expression System

Protocols for 24 and 96 deep well blocks and mini bioreactor tubes

The Gibco™ ExpiCHO™ Expression System brings together a high-expressing CHO cell line and an optimized medium and transfection kit that synergistically act to provide titers as much as 160x higher than the Gibco™ FreeStyle™ MAX CHO Expression System and 4x higher than the Gibco™ Expi293™ Expression System.

The ultrahigh yields of the ExpiCHO Expression System (up to 1–3 g/L for some proteins) allow you to scale down your expression run and achieve significant cost savings compared to other transient expression technologies. Here we present protocols to scale down the system to 24 and 96 deep well blocks and mini bioreactor tubes.

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Transfection in 96 deep well blocks

Materials

- Axygen™ Storage Microplate, Round Bottom, 96 Deep Well (Corning, Cat. No. PDW20CS)
- AeraSeal™ Adhesive Microplate Seals, Sterile (E&K Scientific, Cat. No. T896100-S)
- Thermo Scientific™ Compact Digital Microplate Shaker (Thermo Fisher Scientific, Cat. No. 88880023) or other 3 mm orbital microplate shaker

Note: The use of an orbital shaker is required for this protocol; linear plate shakers do not provide sufficient mixing to support the expression protocol.

Routine subculture

1. Subculture and expand ExpiCHO-S™ cells until the cells reach a density of approximately 4–6 x 10^6 viable cells/mL according to the ExpiCHO kit protocol.

Day –1: Split cells

2. On the day prior to transfection (day –1), split the ExpiCHO-S cells to a final density of 3–4 x 10^6 viable cells/mL and allow the cells to grow overnight according to the ExpiCHO kit protocol.

Day 0: Transfection

3. Dilute cells to 6 x 10^6 viable cells/mL with fresh ExpiCHO™ Expression Medium according to the ExpiCHO kit protocol.

4. Aliquot 0.8 mL of cells into each well of the 96 deep well block (96DWB) to be used for transfection.

Note: The wells on the outer edge of the 96DWB may experience greater evaporation compared to the inner 60 wells. As a precaution to achieve the best well-to-well consistency, we suggest using only the inner 60 wells and adding 1 mL of PBS to the outer wells of the block to minimize the potential for evaporation.

5. Prepare ExpiFectamine™ CHO Reagent–DNA complexes.

   Note: Example volumes below will generate a complexation reaction sufficient to transfect 4 wells. Volumes may be scaled proportionately to transfect other numbers of wells.

   - Dilute plasmid DNA (assuming stock concentration of 1 mg/mL) by adding 2.5 µL DNA to 250 µL Gibco™ OptiPRO™ SFM in a sterile 96-well polypropylene plate and mix by gently pipetting up and down 3–4 times.
   - Add 10 µL ExpiFectamine CHO Reagent to the diluted DNA and mix by gently pipetting up and down 3–4 times.

6. Add 65 µL of the complexation mixture to each well containing culture in the 96DWB and mix by gently pipetting up and down 3–4 times.
   - This results in a final concentration of ~0.8 µg/mL plasmid DNA when added to 0.8 mL of culture volume from step 4. Final plasmid DNA concentrations of 0.6–0.8 µg/mL at this step are typical for most proteins.

7. Cover the plates with a gas-permeable seal.

8. Incubate the 96DWB in a humidified incubator set at 37°C with 8% CO_2 on an orbital shaker (recommended shake speed of 900 rpm for shakers with a 3 mm orbital diameter).

Day 1: Enhancer and feed addition

9. Add ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed 18–22 hours posttransfection.

   Note: All volumes may be scaled proportionately.

   For standard protocol: For the inner 60 wells of a 96DWB, mix 325 µL ExpiFectamine CHO Enhancer and 13 mL ExpiCHO Feed in a conical tube. Add 200 µL of this mixture to each well of the 96DWB. Return 96DWB to the 37°C incubator with 8% CO_2 and shaking.

   For high-titer protocol: For the inner 60 wells of a 96DWB, mix 325 µL ExpiFectamine CHO Enhancer and 13 mL ExpiCHO Feed in a conical tube. Add 200 µL of this mixture to each well of the 96DWB. Transfer 96DWB to a 32°C incubator with 5% CO_2 and shaking.

Days 7–10: Harvest supernatant

10. Due to evaporation, it is recommended to harvest supernatant on the following days posttransfection:

    For standard protocol: Harvest supernatant 7–8 days posttransfection.

    For high-titer protocol: Harvest supernatant 7–10 days posttransfection.

    Results obtained for human IgG expression using both protocols are shown in Figure 1.

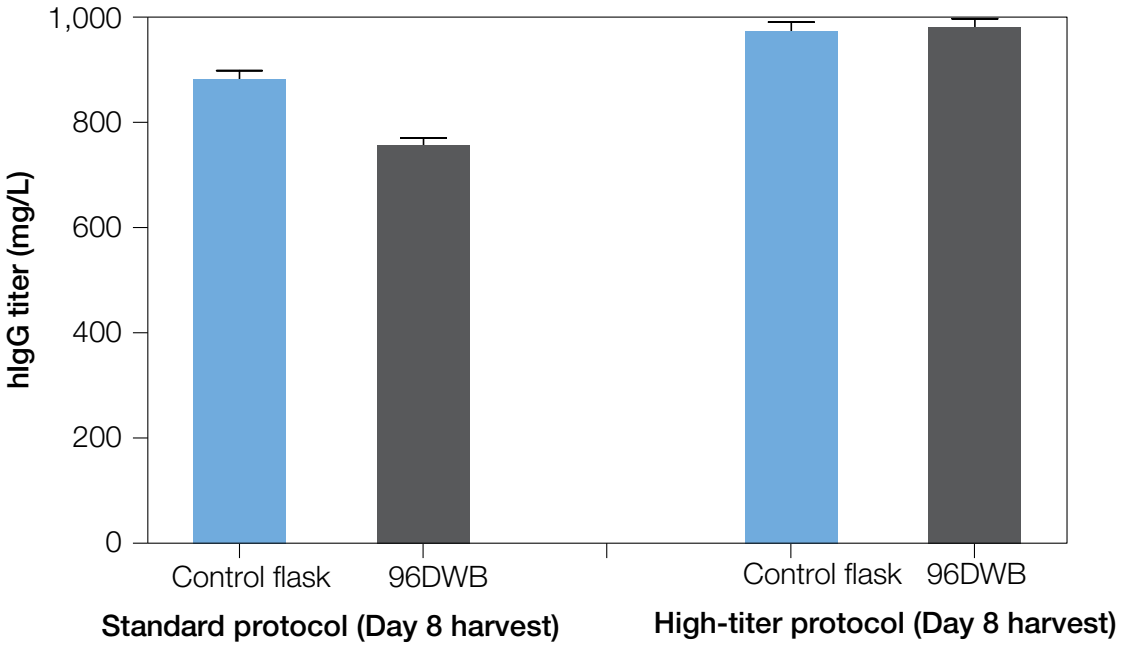

Figure 1. Comparison of human IgG expression using a shaker flask or 96DWB.

Transfection in 24 deep well blocks

Materials

- Axygen™ Storage Microplate, Rectangular Bottom, 24 Deep Well (Corning, Cat. No. PDW10ML24CS)
- AeraSeal™ Adhesive Microplate Seals, Sterile (E&K Scientific, Cat. No. T896100-S)

Note: The shake speed in this protocol has been determined for a shaking platform with a 19 mm orbital diameter. Shake speeds may need to be adjusted for shakers of other diameters.

Routine subculture

1. Subculture and expand ExpiCHO-S cells until the cells reach a density of approximately 4–6 x 10^6 viable cells/mL according to the ExpiCHO kit protocol.

Day –1: Split cells

2. On the day prior to transfection (day –1), split the ExpiCHO-S cells to a final density of 3–4 x 10^6 viable cells/mL and allow the cells to grow overnight according to the ExpiCHO kit protocol.

Day 0: Transfection

3. Dilute cells to 6 x 10^6 viable cells/mL according to the ExpiCHO kit protocol.

4. Aliquot 2.5 mL of cells into each well of the 24 deep well block (24DWB) to be used for transfection.
    - It is recommended to add an equivalent volume of PBS to any non-used wells to minimize evaporation.

5. Prepare ExpiFectamine reagent–DNA complexes.

    Note: Example volumes below will generate complexation reaction sufficient to transfect 1 well. Volumes may be scaled proportionately to transfect other numbers of wells.

    - Dilute plasmid DNA (assuming stock concentration of 1 mg/mL) by adding 2.0 µL DNA to 220 µL OptiPRO SFM for each well to be transfected and mix by gently pipetting up and down 3–4 times.
    - Add 9.0 µL ExpiFectamine CHO Reagent to the diluted DNA and mix by gently pipetting up and down 3–4 times.

6. Add 200 µL of the complexation mixture to each well containing culture in the 24DWB.
    - This results in a final concentration of ~0.8 µg/mL plasmid DNA when added to 2.5 mL of culture volume from step 4. Final plasmid DNA concentrations of 0.6–0.8 µg/mL at this step are typical for most proteins.

7. Cover the plates with a gas-permeable seal.

8. Incubate the 24DWB in a humidified incubator set at 37°C with 8% CO_2 on an orbital shaker (recommended shake speed of 225 rpm for shakers with a 19 mm orbital diameter).

Day 1: Enhancer and feed addition

9. Add ExpiFectamine CHO Enhancer and ExpiCHO Feed 18–22 hours posttransfection.

   Note: All volumes may be scaled proportionately.

   For standard protocol: For an entire 24DWB, mix 400 µL ExpiFectamine CHO Enhancer and 16 mL ExpiCHO Feed in a conical tube. Add 600 µL of this mixture to each well of the 24DWB. Return 24DWB to the 37°C incubator with 8% CO_2 and shaking.

   For high-titer protocol: For an entire 24DWB, mix 400 µL ExpiFectamine CHO Enhancer and 16 mL ExpiCHO Feed in a conical tube. Add 600 µL of this mixture to each well of the 24DWB. Transfer 24DWB to a 32°C incubator with 5% CO_2 and shaking.

Days 7–10: Harvest supernatant

10. Due to evaporation, it is recommended to harvest supernatant on the following days posttransfection:

    For standard protocol: Harvest supernatant 7–8 days posttransfection.

    For high-titer protocol: Harvest supernatant 7–10 days posttransfection.

    Results obtained for human IgG expression using both protocols are shown in Figure 2.

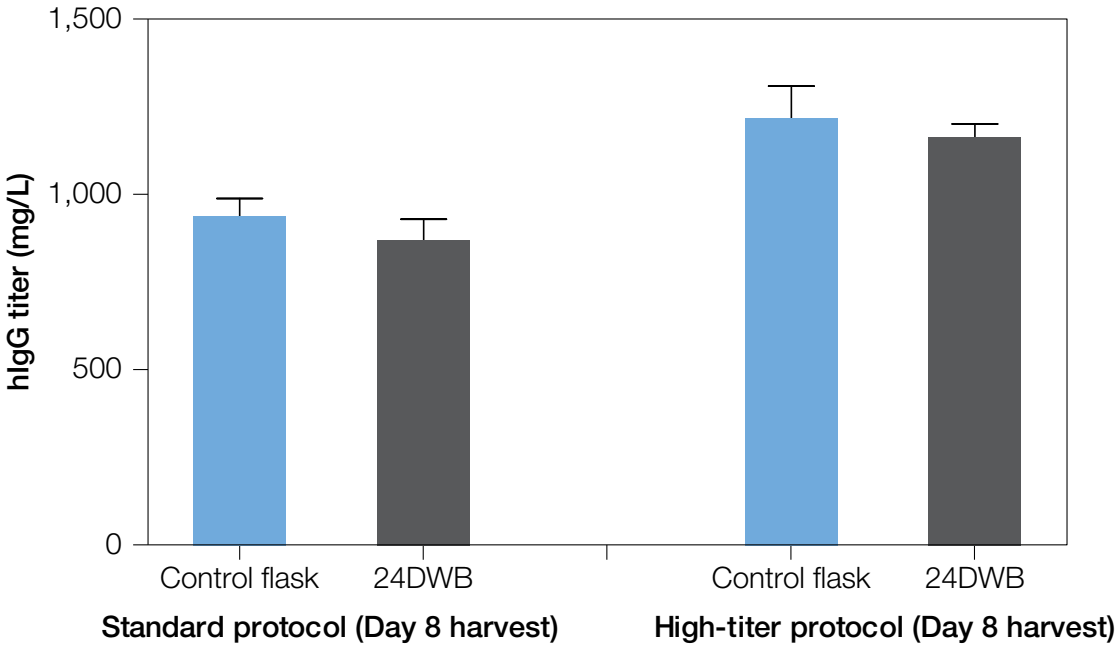

Figure 2. Comparison of human IgG expression using a shaker flask or 24DWB.

Transfection in a 50 mL mini bioreactor

Materials

- Mini bioreactor, 50 mL with hydrophobic vented cap (Corning, Cat. No. 431720)
- Shaking platform with tube rack capable of 45° angle

Note: The shake speed of 240 rpm in this protocol has been determined for a shaking platform with a 19 mm orbital diameter. Shake speeds may need to be adjusted lower for shakers of larger orbital diameters.

Note: The procedures in this protocol are applicable for transfection volumes ranging from 15 to 20 mL. Other volumes will require optimization of shake speed to ensure maximal performance. The example provided below assumes a transfection volume of 15 mL (see step 4). Reagent volumes should be scaled proportionately for transfection volumes other than 15 mL.

Routine subculture

1. Subculture and expand ExpiCHO-S cells until the cells reach a density of approximately 4–6 x 10^6 viable cells/mL according to the ExpiCHO kit protocol.

Day –1: Split cells

2. On the day prior to transfection (day –1), split the ExpiCHO-S cells to a final density of 3–4 x 10^6 viable cells/mL and allow the cells to grow overnight according to the ExpiCHO kit protocol.

Day 0: Transfection

3. Dilute cells to 6 x 10^6 viable cells/mL according to the ExpiCHO kit protocol.
4. Aliquot 15 mL of cells into each mini bioreactor to be used for transfection.
5. Prepare ExpiFectamine reagent–DNA complexes.

   Note: Example volumes below will generate complexation reaction sufficient to transfect 1 mini bioreactor. Volumes may be scaled proportionately to transfect other numbers of mini bioreactors.

   - Dilute plasmid DNA (assuming stock concentration of 1 mg/mL) by adding 12 µL DNA to 1.2 mL OptiPRO SFM for each mini bioreactor to be transfected.
   - Add 50 µL ExpiFectamine CHO Reagent to the diluted DNA and mix by gently pipetting up and down 3–4 times.

6. Add 1.2 mL of the complexation mixture to each mini bioreactor containing ExpiCHO-S cells at 6 x 10^6 viable cells/mL.
   - This results in a final concentration of ~0.8 µg/mL plasmid DNA when added to 15 mL of culture volume from step 4. Final plasmid DNA concentrations of 0.6–0.8 µg/mL at this step are typical for most proteins.
7. Incubate the mini bioreactor in a humidified incubator set at 37°C with 8% CO_2 on an orbital shaker (recommended shake speed of 240 rpm for shakers with a 19 mm orbital diameter). The mini bioreactor should be secured on a 50 mL tube rack set at a 45° angle.

Day 1: Enhancer and feed addition

8. Add ExpiFectamine CHO Enhancer and ExpiCHO Feed 18–22 hours posttransfection.

For standard protocol: For each mini bioreactor, mix 90 µL ExpiFectamine CHO Enhancer and 3.6 mL ExpiCHO Feed in a conical tube, and add all of this mixture to the mini bioreactor. Volumes may be scaled proportionately for other numbers of mini bioreactors. Return mini bioreactor to the 37°C incubator with 8% CO_2 and shaking.

For high-titer protocol: For each mini bioreactor, mix 90 µL ExpiFectamine CHO Enhancer and 3.6 mL ExpiCHO Feed in a conical tube, and add all of this mixture to the mini bioreactor. Volumes may be scaled proportionately for other numbers of mini bioreactors. Transfer mini bioreactor to a 32°C incubator with 5% CO_2 and shaking.

Days 7–10: Harvest supernatant

9. It is recommended to harvest supernatant on the following days posttransfection:

For standard protocol: Harvest supernatant 7–8 days posttransfection.

For high-titer protocol: Harvest supernatant 7–10 days posttransfection.

Results obtained for human IgG expression using both protocols are shown in Figure 3.

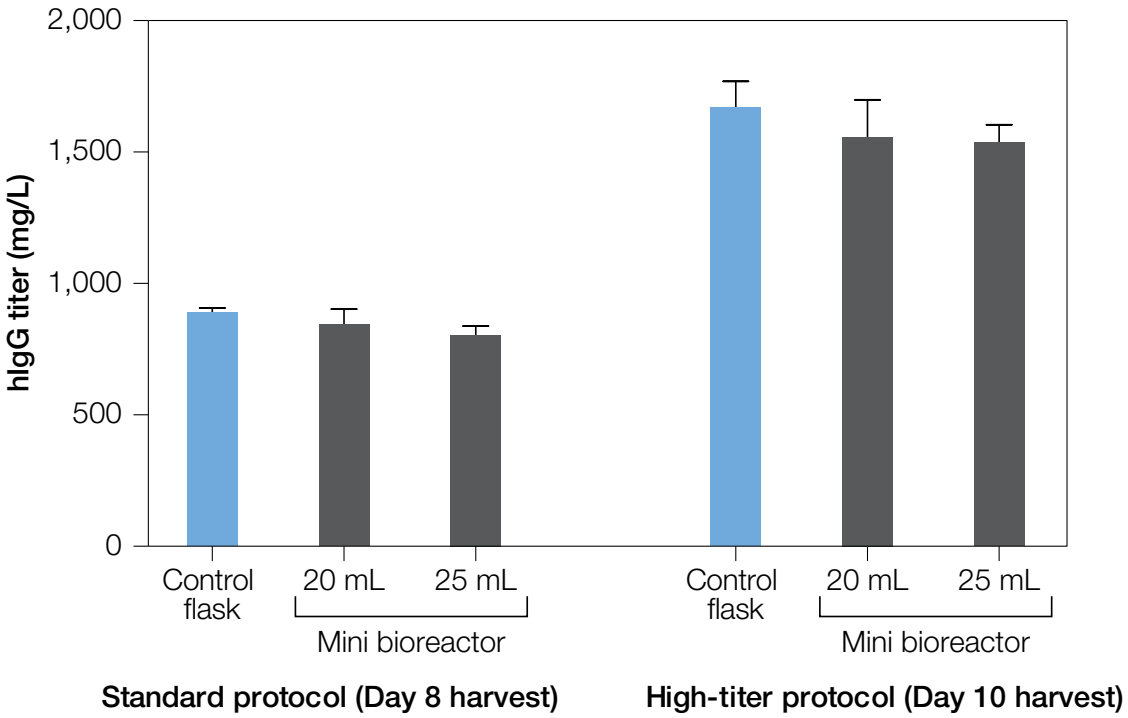

Figure 3. Comparison of human IgG expression using a shaker flask or mini bioreactor with different transfection volumes.

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