# CHOgro® High Yield Expression System

Protocol for MIR 6270

*SDS and Certificate of Analysis available at mirusbio.com/literature*

## INTRODUCTION

The CHOgro® High Yield Expression System is an optimized platform for transient, high titer protein production in suspension CHO derived cells. This system consists of CHOgro® Expression Medium, L-Glutamine and Poloxamer 188 medium supplements, CHOgro® Complex Formation Solution, *Trans*IT-PRO® Transfection Reagent and CHOgro® Titer Enhancer.

CHOgro® Expression Medium is a chemically defined, hydrolysate-free and animal-origin-free medium. CHOgro® Expression Medium is formulated to support high efficiency transfection and provide high density cell growth. In addition, many suspension CHO cells (e.g. Freestyle™ CHO-S and ExpiCHO-S™) can easily grow in CHOgro® Expression Medium with minimal adaptation.

*Trans*IT-PRO® Transfection Reagent was developed by empirically testing proprietary lipid and polymer libraries for high transfection performance in suspension CHO and 293 cell types. The CHOgro® Titer Enhancer was identified and developed for use with the CHOgro® High Yield Expression System to modulate cellular processes and increase protein titers. Both the *Trans*IT-PRO® Reagent and CHOgro® Titer Enhancer are free of animal-derived components and manufactured at Mirus Bio LLC in Madison, Wisconsin, USA.

The combination of CHOgro® Expression Medium, *Trans*IT®-PRO Transfection Reagent and the CHOgro® Titer Enhancer enables robust cell growth and high efficiency transfection that streamlines the transient protein expression process.

The CHOgro® High Yield Expression System ships as multiple components. Store at the temperature listed on the product label.

## SPECIFICATIONS

| | |
|---|---|
| **Storage** | Store *Trans*IT-PRO® Transfection Reagent at -20°C. ***Before each use***, warm to room temperature and vortex gently.<br>Store CHOgro® Titer Enhancer at 2 to 10°C, protected from light.<br>Store CHOgro® Expression Medium and CHOgro® Complex Formation Solution at 4°C, protected from light.<br>Store Poloxamer 188, 10% Solution at room temperature.<br>Store L-Glutamine at -10 to -30°C and avoid multiple freeze/thaw cycles. |
| **Stability/ Guarantee** | *Trans*IT-PRO® Transfection Reagent and CHOgro® Titer Enhancer are guaranteed for 1 year from the date of purchase. Other components are guaranteed as noted on the product label when properly stored and handled. |

**CAUTION:** Standard safe laboratory practices should be maintained when using all chemical transfection reagents. ***Please refer to product SDS for full safety precautions.***

## MATERIALS

### Materials Supplied

The CHOgro® High Yield Expression System (MIR 6270) contains the following components:

| Product No. | Component | Volume |
|---|---|---|
| MIR 5740 | *Trans*IT-PRO® Transfection Reagent | 1 × 1 ml |
| MIR 6220 | CHOgro® Titer Enhancer | 1 × 20 ml |
| MIR 6200 | CHOgro® Expression Medium | 2 × 1000 ml |
| MIR 6210 | CHOgro® Complex Formation Solution | 1 × 100 ml |
| MIR 6230 | Poloxamer 188, 10% Solution | 1 × 100 ml |
| 59202C-100mL | L-Glutamine, 200 mM Solution | 1 × 100 ml |

NOTE: For Materials Required but Not Supplied, see Transfection Protocol (Page 4).

**For Research Use Only**

# Adaptation and Growth in CHOgro® Expression Medium

## Media Preparation

Prior to use, CHOgro® Expression Medium (MIR 6200) requires supplementation with L-Glutamine (4 mM final concentration, 59202C-100mL) and Poloxamer 188 (0.3% final concentration, MIR 6230), as described in Table 1 (below):

**Table 1.** Supplementation required for CHOgro® Expression Medium

| Media Supplements | Per 1000 ml |
|---|---|
| L-Glutamine, 200 mM Solution (59202C-100mL) | 20 ml |
| Poloxamer 188, 10% Solution (MIR 6230) | 30 ml |

*NOTE: Store supplemented media at 4°C, protected from light.*

## Adaptation to CHOgro® Expression Medium:

CHOgro® Expression Medium is a chemically defined, serum-free growth medium that permits high density growth and large-scale transfection of suspension CHO cells. Many suspension CHO cells (e.g. FreeStyle™ CHO-S and ExpiCHO-S™) readily adapt to supplemented CHOgro® Expression Medium through sequential adaptation.

Suspension CHO cells grown in CHOgro® Expression Medium often divide at a faster rate (i.e. doubling every 24 hours) compared to many typical growth media formulations.

### A. Cryopreserved Cell Stock

When bringing suspension CHO cells out of cryopreservation, use supplemented CHOgro® Expression Medium to dilute cells immediately post-thaw, typically at a density of 0.5 - 1 × $10^6$ cells/ml. Incubate cells in a shake flask at an appropriate rpm (e.g. 125 rpm for a 1.9 cm orbital throw) at 37°C, 8% $CO_2$. Monitor cell growth and viability daily. When viability reaches ≥ 98% and the cells are doubling every ≤ 24 hours, the cells are fully adapted.

### B. Ongoing Culture

If cells are cultured in an alternate media formulation, cells must be adapted to CHOgro® Expression Medium prior to transfection with the CHOgro® High Yield Expression System. For adaptation, seed cells at a density of 3 - 5 × $10^5$ cells/ml in a mix of 75% current media and 25% CHOgro® Expression Medium (supplemented with L-Glutamine and Poloxamer) for 2-4 passages or until the cells are doubling normally and viability is > 95%. Increase the ratio of complete CHOgro® Expression Medium in 25% increments and monitor cell health and viability as described above. Cells are fully adapted when viability reaches ≥ 98% and cells are doubling every ≤ 24 hours in 100% CHOgro® Expression Medium. Continue to culture cells in a shake flask at an appropriate rpm (e.g. 125 rpm with a 1.9 cm orbital throw) at 37°C, 8% $CO_2$ and monitor cell growth and viability frequently.

Do NOT allow suspension CHO cells to grow above 1 × $10^7$ cells/ml or below 2.5 × $10^5$ cells/ml during continuous culture.

## Maintenance of Suspension CHO Cells in CHOgro® Expression Medium:

For best results, subculture suspension CHO cells to a density of 1 - 3 × $10^6$ cells/ml. Do NOT allow cells to grow to a density higher than 1 × $10^7$ cells/ml or passage to a density lower than 2.5 × $10^5$ cells/ml during continuous culture. Subculture every 1-4 days to maintain the desired cell density. Monitor cell density and viability frequently (i.e. daily if possible).

# BEFORE YOU START:

## Important Tips for Optimal Plasmid DNA Transfection

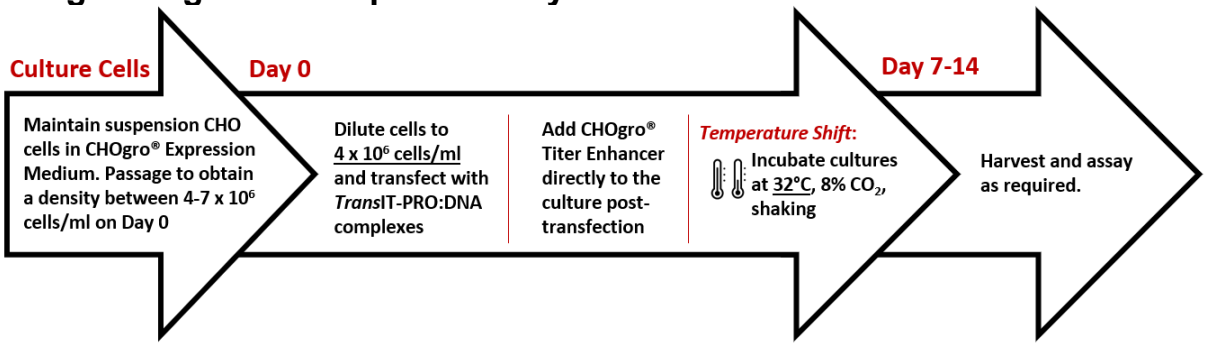
Complete CHOgro® Expression Medium supports high-efficiency transfection and high-density growth of suspension CHO cells. When combined with *Trans*IT-PRO® Transfection Reagent and CHOgro® Titer Enhancer, multi-fold increases in protein titer can be obtained. To ensure successful transfection, we recommend optimizing reaction conditions for each CHO cell subtype.

- **Cell density at transfection.** Cells should be passaged 18-24 hours prior to transfection to obtain a next day density of 4 - 7 × $10^6$ cells/ml. This allows for dilution to a final cell density of **4 × $10^6$ cells/ml** at the time of transfection. For best results, cultures should be maintained at 37°C, 8% $CO_2$ prior to transfection.
- **Cell culture conditions.** Culture cells in CHOgro® Expression Medium supplemented with L-Glutamine (4 mM final concentration, 59202C-100mL) and Poloxamer 188 (0.3% final concentration, MIR 6230) prior to use.
- **Adapting cells to CHOgro®.** Cells grown in alternate media formulations (e.g. ExpiCHO™ Expression Medium) should be fully adapted to CHOgro® Expression Medium supplemented with 4 mM L-Glutamine and 0.3% Poloxamer 188 prior to transfection in the CHOgro® High Yield Expression System. Cells are fully adapted when they are ≥ 98% viable by trypan blue exclusion and doubling normally.
- **DNA purity**. Use highly purified, sterile, endotoxin-free and contaminant-free plasmid DNA for transfection. DNA preparations that have an A260/280 absorbance ratio of 1.8-2.0 are desirable.
- **DNA concentration**. Start with 1 µg of DNA per 1 ml of culture. Vary the DNA concentration from 1-2 µg/ml to find the best working DNA concentration.
- **Ratio of *Trans*IT-PRO® Reagent to DNA.** Start with 1 µl of *Trans*IT-PRO® Reagent per 1 µg of DNA. Vary *Trans*IT-PRO® Reagent from 1-2 µl per 1 µg of DNA to find the optimal ratio.
- **Volume and timing of CHOgro® Titer Enhancer addition.** Add 20 µl of CHOgro® Titer Enhancer per 1 ml of cell culture. CHOgro® Titer Enhancer should be added to the culture immediately after transfection complex addition and prior to incubation at 32°C.
- **Complex formation conditions.** Prepare *Trans*IT-PRO® Reagent:DNA complexes in CHOgro® Complex Formation Solution (MIR 6210). Incubate complexes at room temperature for no more than 5 minutes before adding to cell culture.
- **Feeds.** No feeds are required for high yield, but an optional feed can be added to prolong cellular viability (see 'Addition of Cell Culture Feeds to Extend Cell Viability' on Page 5).
- **Temperature shift.** A temperature shift to 32°C after transfection is *highly recommended* for best results. Cultures can be shifted to 32°C immediately or up to 24 hours post-transfection.
- **Post-transfection incubation time.** The optimal post-transfection incubation time may vary depending on the experimental goal and the nature of the plasmid used. For secreted antibody constructs, optimal titers are obtained at 32°C at 7-14 days post-transfection in batch culture.

Suspension CHO cells grown in CHOgro® Expression Medium often divide at a faster rate compared to other growth media formulations. Passage cells 18-24 hours prior to transfection to obtain a density of 4 - 7 × $10^6$ cells/ml on the day of transfection.

There is no need to perform a media change to remove the transfection complexes. It is beneficial to leave transfection complexes and CHOgro® Titer Enhancer on the cells for the duration of the experiment.

## CHOgro® High Yield Expression System Flow Chart



# TRANSFECTION PROTOCOL

The following procedure describes plasmid DNA transfections in 125 ml Erlenmeyer shake flasks using 20 ml of complete CHOgro® Expression Medium (i.e. supplemented with 4 mM L-Glutamine and 0.3% Poloxamer 188, final concentration). For alternate volumes or cell culture vessels, adjust the amounts of CHOgro® Complex Formation Solution, *Trans*IT-PRO® Transfection Reagent, plasmid DNA and CHOgro® Titer Enhancer based on the volume of complete CHOgro® Expression Medium used in alternate cell culture vessels (see Table 2 for reference).

**Table 2.** Transfection and Enhancer scaling worksheet for CHOgro® High Yield System

| **Starting transfection conditions per milliliter of CHOgro® Expression Medium** | | | | | | |
|---|---|---|---|---|---|---|
| | Per 1 ml | | | Total culture volume | | Reagent quantities |
| CHOgro® Complex Formation Solution | 0.1 | ml | × | _____ml | = | _____ml |
| Plasmid DNA (1 µg/µl stock) | 1 | µl | × | _____ml | = | _____µl |
| *Trans*IT-PRO® Transfection Reagent | 1 | µl | × | _____ml | = | _____µl |
| **Enhancer addition (add directly to culture after transfection complex addition)** | | | | | | |
| CHOgro® Titer Enhancer | 20 | µl | × | _____ml | = | _____µl |

## Materials Required but Not Supplied

- Suspension CHO cells (e.g. FreeStyle™ CHO-S Cells, Gibco™ Cat. No. R800-07, ExpiCHO-S™ Cells, Gibco™ A29-127, or equivalent)
- Erlenmeyer shake flasks (e.g. Corning® Cat. No. 431143 or Thomson Cat. No. 931110)
- Purified, endotoxin-free plasmid DNA
- Sterile tube for transfection complex preparation
- Orbital shaker (e.g. New Brunswick Innova 2000)
- Reporter assay as required

## Transient Plasmid DNA Transfection Protocol for Cells in 125 ml Erlenmeyer Shake Flask (20 ml Culture Volume)

### A. Maintenance of cells

1. Maintain suspension CHO cells in complete CHOgro® Expression Medium (i.e. supplemented with 4 mM L-Glutamine and 0.3% Poloxamer). Passage cells 18-24 hours prior to transfection to ensure cells are actively dividing at the time of transfection and to obtain a density of 4 - 7 × $10^6$ cells/ml the next day.
2. Incubate cells overnight at 37°C in 8% $CO_2$ on an orbital shaker platform.

Passage cells 18-24 hours before transfection to achieve a cell density of 4 - 7 × $10^6$ cells/ml on the day of transfection.

### B. Prepare suspension CHO cells (immediately before transfection)

1. Prior to transfection, count cells and dilute to a density of **4 × $10^6$ cells/ml** into a tissue culture vessel at the desired final volume (e.g. 20 ml per 125 ml Erlenmeyer shake flask). Cultures should be shaking at 37°C in 8% $CO_2$ prior to transfection.

### C. Prepare *Trans*IT-PRO® Reagent:DNA complexes

1. Warm *Trans*IT-PRO® Reagent to room temperature and vortex gently before using.
2. Place 2 ml of CHOgro® Complex Formation Solution in a sterile tube.
3. Add 20 µg plasmid DNA (20 µl of a 1 µg/µl stock). Mix gently but thoroughly.
4. Add 20 µl *Trans*IT-PRO® Reagent to the diluted DNA solution. Mix gently but thoroughly.
5. Incubate at room temperature for no more than 5 minutes.

Incubate complexes for 5 minutes at room temperature prior to adding to cells.

### D. Add transfection complexes and CHOgro® Titer Enhancer to cells

1. Add the *Trans*IT-PRO® Reagent:DNA complexes (prepared in Step C) to culture vessel containing cells in complete CHOgro® Medium (i.e. supplemented with 4 mM L-Glutamine and 0.3% Poloxamer 188). Swirl the flask gently but thoroughly to mix the cells and complexes.
2. Add 400 µl of the CHOgro® Titer Enhancer directly to the culture vessel containing cells and transfection complexes. Swirl the flask gently but thoroughly to mix completely.
   NOTE: CHOgro® Titer Enhancer can be added to the culture at any point from 0-24 hours after addition of transfection complexes.

### E. Immediately perform temperature shift to mild hypothermal conditions

1. Incubate shake flasks on an orbital shaker (125 rpm when using a shaker with a 1.9 cm orbital throw) at 32°C in 8% $CO_2$.
   NOTE: Cells kept at 37°C are generally less productive and experience a decrease in viability at earlier timepoints post-transfection. See 'Extended Viability Protocol' below if prolonged cellular viability is desired.
2. Maintain cultures at 32°C in 8% $CO_2$ for the duration of the expression experiment.
   NOTE: The optimal incubation time will depend on the culture temperature, nature of the expressed protein and detection method. For secreted antibody constructs, optimal titers are typically obtained at 7-14 days post-transfection. Whenever possible, titers should be evaluated prior to 10 days post-transfection, as sufficient titers are often achieved at earlier time points with the CHOgro® High Yield Expression System.
3. Harvest cells and/or supernatant and assay as required.

Place flasks at 32°C immediately after CHOgro® Titer Enhancer addition to increase the specific productivity.

### Addition of Cell Culture Feeds to Extend Cell Viability (Optional)

Maximum yields are achieved if cultures are shifted to mild hypothermal conditions (32°C) immediately after addition of the CHOgro® Titer Enhancer (Step E). Cells maintained at 37°C are generally less productive and experience a decrease in viability at earlier timepoints post-transfection. The following steps can be performed to extend the viability of cells post-transfection for cultures incubated at either 32°C or 37°C.

1. Complete Steps A-D as described in the protocol above.
2. At 24-48 hours post-transfection, add 15% (v/v) culture volume of EX-CELL® Advanced CHO Feed 1 (with glucose) (Sigma Cat. No. 24367C), e.g. 3 ml of EX-CELL® Advanced CHO Feed 1 (with glucose) for a 20 ml culture.
   NOTE: Prepare a fresh solution of EX-CELL® Advanced CHO Feed 1 (with glucose) before each use as the prepared solution is unstable. The optimal amount of feed to add may vary from 10-20% (v/v) culture volume and should be empirically determined for each culture system.
3. Maintain cultures at 32°C (or 37°C if necessary) for the duration of the expression experiment. Whenever possible, monitor cultures for viability and protein titers at several timepoints post-transfection (e.g. 5, 7 and 10 days post-transfection), as sufficient titers are often achieved at earlier time points with the CHOgro® High Yield Expression System.
4. Harvest cells and/or supernatant and assay as required.

Addition of EX-CELL® Advanced CHO Feed 1 (with glucose) is only beneficial for extending cellular viability post-transfection and may not influence protein yield.

# TROUBLESHOOTING GUIDE

## LOW PLASMID DNA TRANSFECTION EFFICIENCY

| Problem | Solution |
|---|---|
| Cells are not fully adapted to CHOgro® Expression Medium | Perform cell counts and evaluate viability daily to ensure that cells are doubling every 24 hours and ≥ 95% viable by trypan blue exclusion. DO NOT proceed with transfection if cells are not doubling normally or are < 98% viable by trypan blue exclusion. |
| | If the cells do not readily adapt to CHOgro® Expression Medium, try a step-wise sequential adaptation protocol. As a general guideline, seed cells at a density of 3–5 × $10^5$ cells/ml in a mix of 75% current and 25% CHOgro® Expression Medium (supplemented with L-Glutamine and Poloxamer 188) for 2-4 passages until the cells return to normal doubling time and viability is > 95%. Do not passage cells if viability is below 95%. Increase the ratio of CHOgro® media (e.g. 50% current and 50% CHOgro® media) stepwise monitoring doubling and viability as outlined above until 100% CHOgro® media is reached. Create a new cell bank in freezing medium (10% DMSO and 90% CHOgro® Expression Medium). |
| Complete growth medium volume too high based on culture vessel size | For standard Erlenmeyer shake flasks, we recommend that the complete growth medium does not exceed one-third the capacity of the flask (e.g. ≤ 40 ml in a 125 ml Erlenmeyer flask). For Thomson Optimum Growth™ flasks the culture volume can be increased per manufacturer's recommendations (e.g. 62 ml in 125 shake flask) without adverse effects on viability or growth. |
| | For spinner flasks, the maximum complete growth medium is equivalent to the capacity of the flask although cell movement and aeration will vary depending on the culture volume. |
| Inhibitor present during transfection | Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-PRO® Reagent:DNA complexes in serum-free growth medium; we recommend CHOgro® Complex Formation Solution. Once transfection complexes are formed, they can be added directly to cells cultured in supplemented CHOgro® Expression Medium. |
| Cells not actively dividing at the time of transfection | Cells should be passaged 18-24 hours prior to transfection to ensure cells are actively dividing at the time of transfection and to obtain a next day density of 4 - 7 × $10^6$ cells/ml. Do NOT proceed with transfections if cells are not doubling daily and > 98% viable.<br>Determine the optimal cell density for each cell type to maximize transfection efficiency. **A final density of 4 × $10^6$ cells/ml for transfection is optimal with the CHOgro® High Yield Expression System.** Cultures should be maintained at 37°C in 8% $CO_2$ prior to transfection. |
| Cultures not shifted to 32°C following addition of transfection complexes and CHOgro® Titer Enhancer | Up to two-fold higher titers are observed with the CHOgro® High Yield Expression System when shifting cultures to mild hypothermal conditions (i.e. to 32°C) post-transfection. Maintenance of cultures at 37°C will lead to lower viabilities at longer timepoints due to depletion of nutrients. A temperature shift to 32°C is STONGLY RECOMMENDED. |

# TROUBLESHOOTING GUIDE continued

| LOW PLASMID TRANSFECTION EFFICIENCY | |
|---|---|
| **Problem** | **Solution** |
| Low-quality plasmid DNA | Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. |
| | We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation.<br>Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells. |
| | Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin. |
| *Trans*IT-PRO® Reagent was not mixed properly | Warm *Trans*IT-PRO® Reagent to room temperature and vortex gently before each use. |
| Suboptimal *Trans*IT-PRO® Reagent:DNA ratio | Determine the best *Trans*IT-PRO® Reagent:DNA ratio for each cell type. Titrate the *Trans*IT-PRO® Reagent from 1-2 µl per 1 µg DNA. Refer to "Before You Start" on Page 3. |
| Suboptimal CHOgro® Titer Enhancer volume added to culture | Typically, 20 µl CHOgro® Titer Enhancer per milliliter of culture is optimal. CHOgro® Titer Enhancer can be added to cultures between 0-24 hours after transfection. We recommend adding CHOgro® Titer Enhancer immediately post-transfection for the most streamlined workflow. |
| Suboptimal DNA concentration | Confirm DNA concentration and purity. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8-2.0. |
| | The optimal DNA concentration generally ranges between 0.5-2.0 µg/ml of culture medium. Start with a DNA concentration of 1 µg/ml. Consider testing more or less DNA while scaling the amount of *Trans*IT-PRO® Transfection Reagent accordingly. |
| Incorrect vector sequence | If no expression of your target insert is observed, verify the sequence of the plasmid DNA. |
| Post-transfection harvest time | Determine the optimal transfection incubation time for each cell type and experiment. The optimal incubation time will vary depending on the goal of the experiment and the nature of the plasmid used. For secreted antibody constructs, **optimal titers are typically obtained at 32°C at 7-14 days post-transfection in batch culture**. For 37°C cultures (i.e. cultures not shifted to 32°C post-transfection), shorter incubation times of 5-7 days are recommended due to decreased cell viability from depletion of culture nutrients. |
| Precipitate formation during transfection complex formation | Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. As recommended in the protocol, always dilute DNA first in CHOgro® Complex Formation Solution and mix before adding *Trans*IT-PRO® to the diluted DNA mixture during complex formation. |
| Proper experimental controls were not included | To verify efficient transfection, use *Trans*IT-PRO® Transfection Kit to deliver a positive control such as the Human IgG1 Expression Control (MIR 6250), luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid. |
| | To assess delivery efficiency of plasmid DNA, use the Mirus Bio *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid, or use the prelabeled *Label* IT® Plasmid Delivery Controls (please refer to Related Products on Page 9). |

# TROUBLESHOOTING GUIDE continued

| HIGH CELLULAR TOXICITY | |
|---|---|
| **Problem** | **Solution** |
| Shake/ spin culture conditions not optimal | Excessive agitation is harmful to cells. Monitor cell viability using trypan blue exclusion. |
| Cells not properly adapted to growth culture medium prior to transfection | Check the viability of cultured cells before transfection. Ensure complete adaptation to growth culture medium by verifying consistent doubling times and viability ≥ 98% using trypan blue exclusion over several days up to one week. |
| Cell density not optimal at time of transfection | Determine the best cell density for each cell type to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For best results with most suspension CHO cell types, **a density of 4 × $10^6$ cells/ml is recommended** at the time of transfection with the CHOgro® High Yield Expression System. Use of higher or lower densities may be desirable depending on cell type, length of experiment and feeding schedule. |
| CHOgro® Expression Medium was not properly supplemented | Prior to use, CHOgro® Expression Medium (MIR 6200) requires supplementation with L-Glutamine (4 mM final concentration, 59202C-100mL) and Poloxamer 188 (0.3% final concentration, MIR 6230). |
| Cell morphology has changed | Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check your cells for mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate Mycoplasma.<br>A high or low cell passage number can make cells more sensitive and/or refractory to transfection. Maintain a similar passage number between experiments to ensure reproducibility. |
| Endotoxin-contaminated plasmid DNA | Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection.<br>We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells.<br>Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin. |
| Expressed target gene is toxic to cells | Compare toxicity levels against a cells alone control and cells transfected with an empty vector to assess the cytotoxic effects of the target protein being expressed.<br>If lower levels of target gene expression are desired in your transfection experiments, consider reducing the amount of target plasmid. Maintain the optimal *Trans*IT-PRO® Reagent:DNA ratio by using carrier DNA such as an empty cloning vector. |
| Cultures not shifted to 32°C following addition of transfection complexes and CHOgro® Titer Enhancer | Maximum yields are achieved if cultures are shifted to mild hypothermal conditions (32°C) immediately after addition of the CHOgro® Titer Enhancer. Cells maintained at 37°C are generally less productive and experience a decrease in viability at earlier timepoints post-transfection.<br>See '**Addition of Cell Culture Feeds to Extend Cell Viability**' on Page 5. |

# RELATED PRODUCTS

- CHOgro® Transfection and Titer Enhancer Kit
- CHOgro® Expression Medium, Dry Powder (10L)
- CHOgro® Expression Medium, 10 L Polybag
- Human IgG1 Expression Control
- *Trans*IT-PRO® Transfection Kit
- MiraCLEAN® Endotoxin Removal Kits
- *Label* IT® Nucleic Acid Labeling Kits
- Ingenio® Electroporation Solution and Kits

For details on the above-mentioned products, visit www.mirusbio.com

**Reagent Agent®**

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

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www.mirusbio.com/ra

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