CHOgro® Transfection and Titer Enhancer Kit

Quick Reference Protocol

Instructions for MIR 6225

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6225

SPECIFICATIONS

Storage	Store *Trans*IT-PRO® Transfection Reagent (MIR 5740) tightly capped at -20°C. Store CHOgro® Titer Enhancer (MIR 6220) at 2-10°C, protected from light. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.
Intended Usage	Designed for use with CHOgro® High Yield Expression System (MIR 6270)

PLASMID DNA TRANSFECTION PROTOCOL

Full protocol and additional documentation available at ***mirusbio.com/6225***

CHOgro® High Yield Expression System Flow Chart

A. Culture Cells
Maintain suspension CHO cells in CHOgro® Medium. Passage to obtain next-day density of 4-7 x 10^6 cells/ml.

Day 0

B. Dilute cells to 4 x 10^6 cells/ml

C. Prepare transfection complexes. Incubate at RT for 5 minutes.

D. Add complexes and CHOgro® Titer Enhancer to cell culture

E. *Temperature shift:*

Incubate cultures at 32°C, 8% CO_2, shaking

Day 7-14
Harvest and assay as required

Fill in volumes below based on total culture volume (Table 1).

A. Maintenance of cells
1. Passage cells 18–24 hours prior to transfection to obtain a next day density of 4-7 x 10^6 cells/ml. DO NOT proceed with transfection if cells are not doubling every 24 hours or are < 98% viable.
2. Incubate cells overnight at 37°C in 8% CO_2, shaking.

B. Prepare/dilute suspension CHO cells for transfection
1. Seed cells at 4 x 10^6 cells/ml immediately before transfection. DO NOT proceed if cells are not doubling normally or < 98% viable.

C. Prepare *Trans*IT-PRO® Reagent:DNA complexes
1. Warm *Trans*IT-PRO® Reagent to room temperature. Vortex gently.
2. Place ___ml CHOgro® Complex Formation Solution in sterile tube.
3. Add ___µg plasmid DNA. Mix gently by pipetting.
4. Add ___µl of *Trans*IT-PRO® Reagent. Mix gently by pipetting.
5. Incubate at room temperature for no more than 5 minutes to allow transfection complexes to form.

D. Add transfection complexes and CHOgro® Titer Enhancer to cells
1. Add transfection complexes to cell culture. Mix gently by swirling.
2. Add ___µl of CHOgro® Titer Enhancer to cell culture + transfection complexes. Mix gently by swirling.

E. Place culture at 32°C after transfection and enhancer addition
1. Incubate cultures at 32°C (8% CO_2, shaking) for 2-14 days. NOTE: Optimal culture time will depend on cell type, protein of interest, culture temperature, and detection method.
2. Harvest cells and/or supernatant and assay as required.

Table 1. Scaling worksheet for CHOgro® Transfection and Titer Enhancer Kit.

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

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Critical Parameters for Success with CHOgro® High Yield Expression System

- **Cell adaptation and maintenance.** Cells grown in alternate media formulations should be fully adapted to CHOgro® Expression Medium supplemented with 4mM L-Glutamine and 0.3% Poloxamer 188 prior to transfection with the CHOgro® High Yield Expression System. Cells are fully adapted when they are ≥98% viable and doubling normally.
- **Cell density at transfection.** Cells should be passaged 18–24 hours prior to transfection to obtain a next day density of 4-7 x 10^6 cells/ml. This allows for a minimal cell dilution for a final density of 4 x 10^6 cells/ml at the time of transfection. Cultures should be placed at 37°C in 8% CO_2 prior to transfection. DO NOT proceed with transfection if cells are not doubling daily and at least 98% viable by trypan blue exclusion.
- **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. To maintain the *Trans*IT-PRO® Reagent:DNA ratio, adjust the reagent volume accordingly. NOTE: Use only high quality, endotoxin-free DNA for transfections. Contaminants such as protein, carbohydrate and lipids may affect transfection efficiency and gene expression levels. Ensure that the plasmid preparation exhibits an A260/A280 ratio of > 1.8.
- **Ratio of *Trans*IT-PRO® Reagent to DNA**. Start with 1 µl of *Trans*IT-PRO® Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-PRO® Reagent from 1–2 µl per 1 µg of DNA to find the optimal ratio.
- **Transfection complex formation**. 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 cultures directly.
- **CHOgro® Titer Enhancer addition**. CHOgro® Titer Enhancer should be added to the culture immediately after transfection complex addition. Add 20 µl of CHOgro® Titer Enhancer per 1 ml cell culture (see Table 1 on front page for scaling chart). Cultures should then be placed at 32°C, 8% CO_2 (shaking) for the remainder of the culture.
- **Feeds.** No feeds are required for high yield, but an optional feed can be added to prolong cellular viability (see CHOgro® High Yield Expression protocol for details).
- **Temperature shift to 32°C post-transfection.** Placing flasks at 32°C immediately post-transfection will increase overall protein titers and decrease protein degradation. Typically, greater than 2-fold higher antibody titers are achieved if incorporating the temperature shift into the production workflow.
- **Post-transfection incubation time.** The optimal post-transfection incubation time may vary by the experimental goal and the plasmid used. For secreted antibody constructs, optimal titers are obtained at 32°C at 7-14 days post-transfection in batch culture.

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Rev.B 050120