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

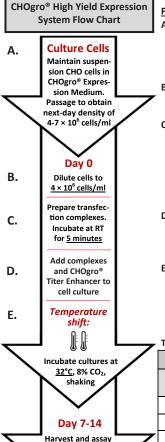


SPECIFICATIONS

Storage	Store <i>Trans</i> 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	oduct 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





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 \times 10⁶ 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% CO₂, shaking.

B. Dilute suspension CHO cells for transfection

Seed cells at 4 × 10⁶ cells/ml immediately before transfection.
 DO NOT proceed if cells are not doubling normally or < 98% viable.

C. Prepare TransIT-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 <u>5 minutes</u> 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

- Incubate cultures at 32°C (8% CO₂, 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 μΙ	×	ml	=	μΙ		
TransIT-PRO® Reagent	1 μΙ	×	ml	=	μΙ		
Enhancer addition (add to culture after transfection complex addition):							
CHOgro® Titer Enhancer	20 μΙ	×	ml	=	μΙ		

▶ 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 4 mM 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 × 10⁶ cells/ml. This allows for a minimal cell dilution for a final density of 4 × 10⁶ cells/ml at the time of transfection. Cultures should be placed at 37°C in 8% CO₂ 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 μ I of *Trans*IT-PRO® Reagent per 1 μ g of DNA. Vary the concentration of *Trans*IT-PRO® Reagent from 1-2 μ I per 1 μ g of DNA to find the optimal ratio.
- Transfection complex formation. Prepare TransIT-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% CO₂ (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.



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.

Learn more at: mirusbio.com/ra

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