

# 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](http://mirusbio.com/6225)



### SPECIFICATIONS

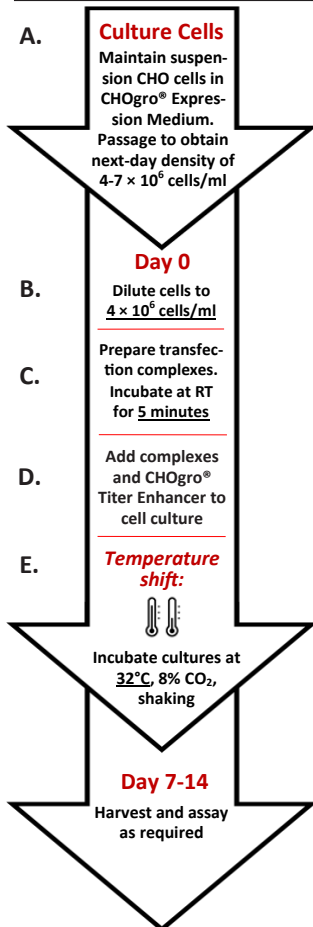
Storage	Store <i>TransIT-PRO</i> ® Transfection Reagent (MIR 5740) tightly capped at -20°C. Store CHOgro® Titer Enhancer (MIR 6220) at 2-10°C, protected from light. <b>Before each use</b> , warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored 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](http://mirusbio.com/6225)

#### CHOgro® High Yield Expression System Flow Chart



#### 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  $4-7 \times 10^6$  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<sub>2</sub>, shaking.

##### B. Dilute suspension CHO cells for transfection

1. Seed cells at  $4 \times 10^6$  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 *TransIT-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 *TransIT-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<sub>2</sub>, 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
<i>TransIT-PRO</i> ® 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 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  $\geq 98\%$  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  $4-7 \times 10^5$  cells/ml. This allows for a minimal cell dilution for a final density of  $4 \times 10^6$  cells/ml at the time of transfection. Cultures should be placed at  $37^\circ\text{C}$  in  $8\% \text{CO}_2$  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  $1 \mu\text{g}$  of DNA per 1 ml of culture. Vary the DNA concentration from  $1-2 \mu\text{g}/\text{ml}$  to find the best working DNA concentration. To maintain the *TransIT-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 *TransIT-PRO*® Reagent to DNA.** Start with  $1 \mu\text{l}$  of *TransIT-PRO*® Reagent per  $1 \mu\text{g}$  of DNA. Vary the concentration of *TransIT-PRO*® Reagent from  $1-2 \mu\text{l}$  per  $1 \mu\text{g}$  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  $20 \mu\text{l}$  of CHOgro® Titer Enhancer per 1 ml cell culture (see Table 1 on front page for scaling chart). Cultures should then be placed at  $32^\circ\text{C}$ ,  $8\% \text{CO}_2$  (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  $32^\circ\text{C}$  post-transfection.** Placing flasks at  $32^\circ\text{C}$  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  $32^\circ\text{C}$  at 7-14 days post-transfection in batch culture.



### Reagent Agent®

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