CHOgro® Transfection and Titer Enhancer Kit

Quick Reference Protocol

Instructions for MIR 6225

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

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.
Usage	Designed for use with CHOgro® High Yield Expression System (MIR 6270), see full protocol.

▸ PLASMID DNA TRANSFECTION PROTOCOL

Full protocol and additional documentation available at mirusbio.com/literature

Prior to Transfection

Maintain Cells

Ensure cells are >98% viable and doubling every 24 h.

1. Passage cells 18-24 h prior to seeding to achieve a cell density of 4-7×10^6 cells/ml and incubate overnight at 37°C and 8% CO_2, shaking.

Day 0

Seed Cells & Prepare Transfection Complexes

2. Seed cells at a density of 4×10^6 cells/ml on the day of transfection.
3. Prepare transfection complexes in a sterile tube using volumes in **Table 1**, in the order below:
   a. Add DNA to the indicated volume of CHOgro® Complex Formation Solution or PBS and vortex.
   b. Add *Trans*IT-PRO® Transfection Reagent and vortex.
   c. Incubate for <5 mins at RT. Do not vortex after the incubation period.

Transfect & Shift Temperature

4. Add transfection complexes to culture and swirl gently, followed immediately by the CHOgro® Titer Enhancer. Swirl to mix.
5. Move cultures to 32°C incubator (8% CO_2, shaking).

Day 2 - 14

Harvest

Optimal post-transfection incubation times will vary.

6. Harvest protein 2-14 days after transfection.

Table 1: Scaling Sheet for CHOgro® Transfection and Titer Enhancer Kit

Culture Volume	*Per 1 ml*	25 ml	100 ml	2 L
CHOgro® Complex Formation Solution or PBS	*100 µl*	2.5 ml	10 ml	200 ml
Plasmid DNA (1 µg/µl stock)	*1 µl*	25 µl	100 µl	2 ml
*Trans*IT-PRO® Transfection Reagent	*1 µl*	25 µl	100 µl	2 ml
After Transfection Complex Addition				
CHOgro® Titer Enhancer	*20 µl*	500 µl	2 ml	40 ml

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Important Factors to Consider

- **Cell adaptation and maintenance.** Cells should be fully adapted to the media they are grown in, such as CHOgro® Expression Medium supplemented with 4 mM L-Glutamine and 0.3% (w/v) Poloxamer 188 (30 ml/L of culture if using 10% (w/v) stock solution). Cells are fully adapted when they are ≥ 98% viable and doubling every 24 hours.
- **DNA concentration.** Start with 1 µg of DNA per 1 ml of culture, which can be optimized to 1-2 µg/ml of culture. Use only high quality, endotoxin-free DNA for transfections. Ensure that the plasmid preparation has 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. If necessary, 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**. *Trans*IT-PRO® Reagent:DNA complexes can be prepared in CHOgro® Complex Formation Solution (MIR 6210) or PBS. Incubate complexes at room temperature for no more than 5 minutes after mixing. Do not vortex after the incubation step.
- **CHOgro® Titer Enhancer addition**. CHOgro® Titer Enhancer should be added to the culture immediately after transfection complex addition.
- **Feeds.** Feeds are not required, but can be added to prolong cellular viability (see CHOgro® High Yield Expression System Full Protocol for details), such as EX-CELL® Advanced CHO Feed 1 with glucose (Millipore Sigma Cat No. 24367C).
- **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, 7-14 days post-transfection in batch culture.

Reagent Agent®

Unsure which transfection reagent is best for you? Consult Reagent Agent™.
mirusbio.com/ra

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