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



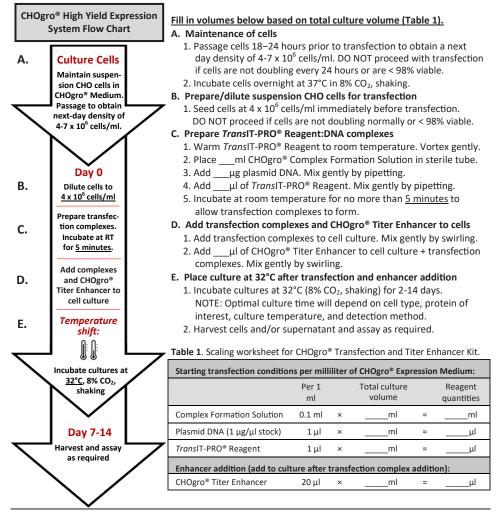
SPECIFICATIONS

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. <i>Before each use</i> , 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



▶ 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 10⁶ cells/ml. This allows for a minimal cell dilution for a final density of 4 x 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 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 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 <u>CHOgro® High Yield Expression protocol</u> for details).
- **Temperature shift to 32°C post-transfection.** Placing flasks at 32°C immediately posttransfection 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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