

Maximize Protein Expression in CHO Suspension Cells

Reach Critical Stages Faster: High Protein Yield and No Medium Change

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Transient transfection in mammalian cell lines provides an avenue for researchers to bridge the development bottleneck and shorten the time to usable protein. The method also maintains post translational modifications crucial for biotherapeutic function. Chinese hamster ovary (CHO) suspension cells are especially suited for high-yield production of recombinant proteins, despite being refractory to commonly used transfection methods (e.g., 25-kDa linear PEI). Mirus Bio has developed a more effective alternative, the *TransIT-PRO*[™] transfection kit. Using this kit increases yields of recombinant protein that can be obtained with minimal optimization of transfection parameters. Transfection complexes are made in serum-free media by adding plasmid DNA, *TransIT-PRO* Transfection Reagent, and the PRO Boost Reagent (optional). After incubating complexes for 15–30 minutes, they can be added directly to cells in normal growth media. Transfection using the *TransIT-PRO* Transfection Kit eliminates the need for a culture medium change post-transfection and is suitable for both transient and stable transfection. For secreted antibody constructs, optimal titers are typically obtained five to seven days post-transfection in batch fermentation.

To demonstrate biotherapeutic relevant protein production a representative human IgG1 was expressed in CHO suspension cells using multiple transfection methods including: *TransIT-PRO*[™] Transfection Kit, 25-kDa linear PEI or FreeStyle[™] Max Transfection Reagent. Figure 1 demonstrates expression of a human IgG1 construct by Western blot using supernatants collected at day 7 post-transfection using FreeStyle[™] CHO-S cells grown in FreeStyle[™] CHO medium. In this system, the *TransIT-PRO* Transfection Kit and FreeStyle Max Transfection Reagent perform similarly, outperforming 25-kDa linear PEI by three- to five-fold.

MAKE PROTEIN PRODUCTION TURNKEY

TransIT-PRO is Compatible with Multiple CHO Media

Formulations: Cell culture growth medium has a considerable influence on transfection efficiency. To further examine these effects, FreeStyle CHO-S cells were adapted to five representative growth media including BD Select[™] CD1000



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Figure 1: Achieve high antibody titers using the *TransIT-PRO*[™] Transfection Kit. Human IgG1 was produced by transient transfection using *TransIT-PRO* and PRO Boost Reagent (1:1:1) (Mirus Bio, Madison, WI), 25 kDa linear PEI (6:1) (Polysciences, Warrington, PA), or FreeStyle[™] Max (1:1) (Life Technologies Corporation, Carlsbad, CA) transfection reagents according to the manufacturers or published protocol (reagent:DNA ratio). Transfections were performed using 1 µg plasmid DNA per milliliter of culture and 0.5 x 10⁶ cells/mL at the time of transfection. FreeStyle[™] CHO-S cells were cultured in 20mL of FreeStyle[™] CHO Expression medium (Life Technologies Corporation, Carlsbad, CA) in 125 ml shake flasks. Day 7 supernatants were clarified and analyzed by Western blot. An IgG standard was included for quantification estimate (S3 = 6.3 mg/L, S2= 3.2 mg/L, S1= 1.6 mg/L).



medium, BD Select[™] CHO medium, FreeStyle CHO expression medium, PowerCHO[®]2 CD medium, and ProCHO[™]5 medium. Cells adapted to the appropriate media were transfected with a plasmid encoding a human IgG1 antibody construct using multiple transfection methods including: *TransIT-PRO* transfection kit, 25-kDa linear PEI, and FreeStyle Max transfection reagent (Figure 2). Clarified supernatants were analyzed using a human IgG-Fc sandwich ELISA. The media formulation impacted transfection efficiencies by >10-fold within a given transfection methodology.

Not all commercially available transfection reagents had broad-spectrum media compatibility. At least a 10-fold increase in IgG yield was obtained when the *TransIT-PRO* Transfection Kit was used in conjunction with FreeStyle CHO-S cells adapted to BD-Select CD1000 medium (Figure 2, red bars). Similar to Figure 1, comparable results were obtained with the *TransIT-PRO* Transfection Kit and FreeStyle Max with CHO-S cells adapted to FreeStyle CHO medium (Figure 2, blue bars). Notably, in many

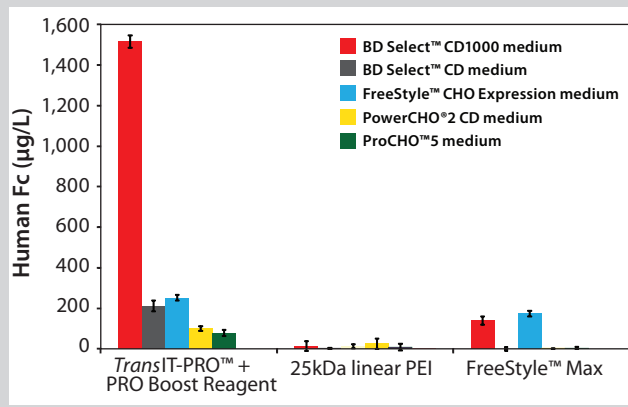
media formulations (BD Select CHO, ProCHO5 and PowerCHO2 CD) the *TransIT-PRO* Transfection Kit provided detectable levels of expression, significantly outperforming 25-kDa linear PEI and FreeStyle Max transfection reagent.

SCALE-UP: NO PROBLEM

These results demonstrate that high protein titers can be obtained with transient transfection using the *TransIT-PRO* Transfection Kit and many chemically defined media formulations. Transfections are scalable from 2-mL to 400-mL culture volumes (data not shown). The *TransIT-PRO* Transfection Kit provides an alternative to FreeStyle Max reagent and outperforms linear PEI, saving valuable time and research costs. *TransIT-PRO* uses components free of animal origin designed for high and reproducible protein production in suspension CHO cells. 🌐

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Figure 2: *TransIT-PRO* provides high performance across varied media formulations. CHO-S cells were adapted to five representative growth media including: BD Select™ CD1000 medium (Becton, Dickinson and Company, Franklin Lakes, NJ), BD Select™ CHO medium (Becton, Dickinson and Company, Franklin Lakes, NJ), FreeStyle™ CHO Expression medium (Life Technologies Corporation, Carlsbad, CA), ProCHO™5 medium (Lonza Inc., Allendale, NJ) and PowerCHO™2 CD medium (Lonza Inc., Allendale, NJ). Cells were transfected with a plasmid encoding a human IgG1 construct using the *TransIT-PRO* and PRO Boost Reagent (1:1:1), 25 kDa linear PEI (4:1), or FreeStyle Max (1:1) transfection reagents according to the manufacturers or published protocol (reagent:DNA ratio). Transfections were performed using 2-mL culture volume in 24-well deep well shaker blocks with 1 µg plasmid DNA per mL of culture and 0.5×10^6 cells/mL at the time of transfection. Human IgG1 was quantitated from day 5 clarified supernatants and analyzed by a standard sandwich ELISA. Error bars represent the standard deviation of triplicate wells.



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