Maximizing protein expression in CHO suspension cells through transient transfection

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Abstract

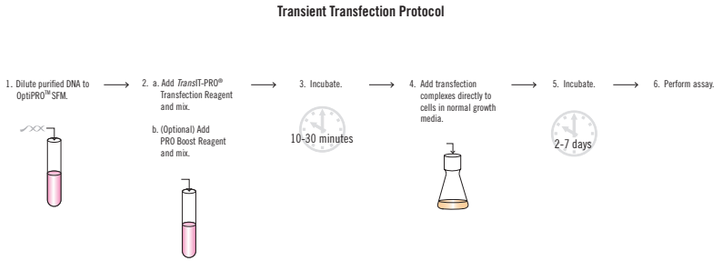
Transient transfection in mammalian cell lines provides an avenue for researchers to bridge the development bottleneck and shorten the time to usable protein. The method also maintains post translational modifications crucial for biotherapeutic function. Chinese Hamster Ovary (CHO) suspension cells are especially suited for high yield production of recombinant proteins, despite being refractory to commonly used transfection methods (e.g. 25kDa linear PEI). Mirus Bio has developed a more effective alternative, the *Trans*IT-PRO® Transfection Kit.

Transfection efficiencies are affected by many parameters including cell density at the time of transfection, DNA concentration and reagent dosing. Based on extensive testing performed during product development and the data presented herein our starting recommendations include an initial cell density of 0.5 - 1.0 x 10^6 cells/ml, 1 µg of DNA per milliliter of culture volume and *Trans*IT-PRO:PRO Boost Reagent:DNA ratio of 1:0.5:1. Optimization of transfection parameters will maximize transfection efficiency and reduce the impact of variables such as differences in cell lineage, handling and media formulation.

Cell culture growth medium has a considerable influence on transfection efficiency. To further examine these effects, suspension CHO cells were adapted to five representative growth media, transfected using multiple methods and analyzed for efficiency by luciferase reporter assay or IgG titer. The media formulation impacted transfection efficiencies by greater than 10-fold within a given transfection methodology. In addition, not all commercially available transfection reagents had broad spectrum media compatibility. A 10-fold increase in IgG yield was obtained when the *Trans*IT-PRO Transfection Kit was used in conjunction with suspension CHO cells adapted to BD-Select™ CD1000 medium. Notably, in many media formulations (BD Select™ CHO, ProCHO™5 and PowerCHO®2 CD) the *Trans*IT-PRO Transfection kit provided detectable levels of antibody production, significantly outperformed 25kDa linear PEI and FreeStyle™ Max Transfection Reagent.

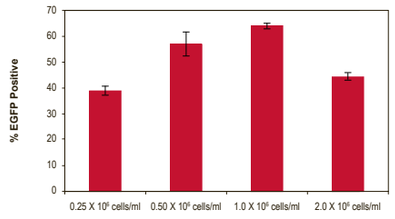
The *Trans*IT-PRO Transfection Kit uses animal origin free components designed for high and reproducible protein yield in suspension CHO cells. Transfections using the *Trans*IT-PRO Transfection Kit are linear in culture volumes ranging from 4-400 milliliters in shake or spinner vessels. Competitor benchmarking experiments were performed utilizing the FreeStyle Max expression system and multiple transfection methods. Western blot and ELISA detecting human IgG levels demonstrate that the *Trans*IT-PRO Transfection Kit and FreeStyle Max Transfection Reagent perform similarly, outperforming 25 kDa linear PEI by 5-fold. The results demonstrate that maximum levels of transient expression are achieved when an effective delivery reagent is combined with optimized transfection parameters and growth conditions.

Method Schematic



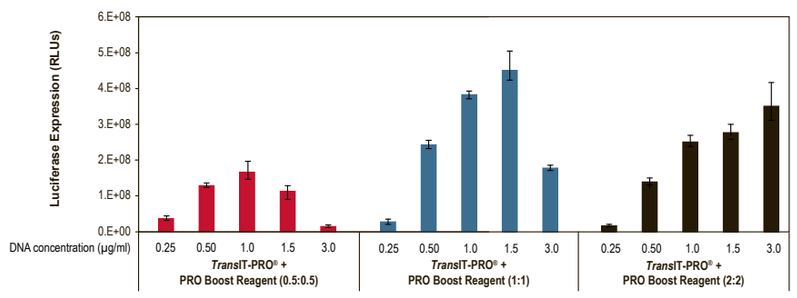
***Trans*IT-PRO Transfection Protocol.** Transfection complexes are made in serum-free media by adding plasmid DNA, *Trans*IT-PRO Transfection Reagent, PRO Boost Reagent (optional) and mixing gently. After incubating complexes for 10–30 minutes they can be added directly to cells in normal growth media. Transfection using the *Trans*IT-PRO Transfection Kit eliminates the need for a culture medium change post-transfection and is suitable for both transient and stable transfection. Incubate 2–7 days post-transfection depending on the goal of the experiment and the nature of the plasmid used.

Protocol Optimization - Cell Density



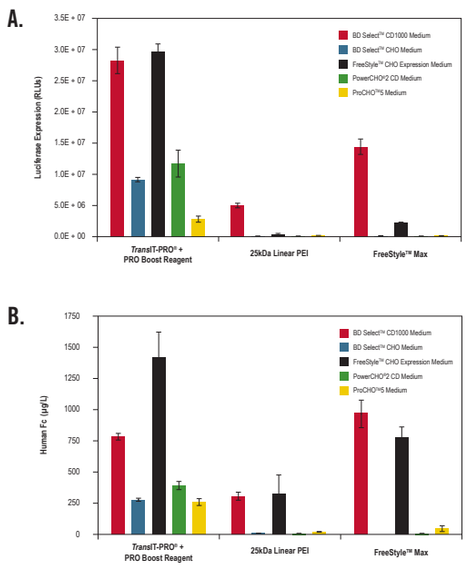
Optimal cell density at the time of transfection is 0.5 - 1.0 x 10^6 cells/ml. CHO-S cells were transfected using the *Trans*IT-PRO® Transfection Kit (Mirus Bio, Madison, WI) at a range of cell densities (0.25 - 2.0 x 10^6 cells/ml) at the time of transfection. Complexes were formed at a 1:1:1 ratio of *Trans*IT-PRO:Boost Reagent:DNA using an enhanced green fluorescence protein (EGFP) reporter plasmid. GFP efficiency was determined at 48 hours post-transfection using flow cytometry. Dead cells were excluded from analysis by propidium iodide staining. Transfections were performed in 24-well deep well shaker blocks using FreeStyle™ CHO-S cells cultured in FreeStyle™ CHO Expression media (2ml/well). Error bars represent the standard deviation of triplicate wells.

Protocol Optimization - DNA and Reagent Dosing



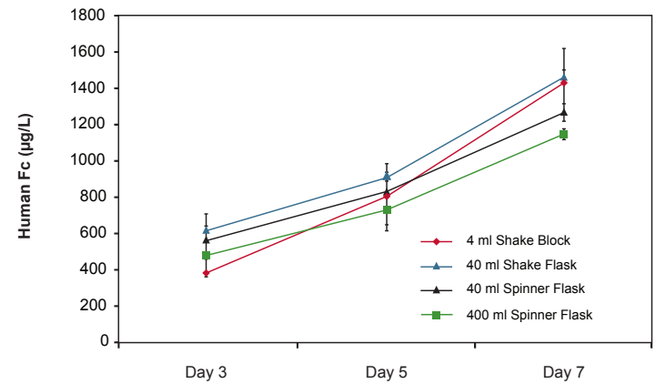
DNA concentrations of 1-1.5 µg per milliliter of culture combined with a *Trans*IT-PRO® and PRO Boost Reagent ratio of 1:1 demonstrate high performance. Luciferase protein expression was compared at varying plasmid DNA concentrations (0.25 - 3 µg/ml) and using three different ratios of *Trans*IT-PRO:PRO Boost Reagent (0.5:0.5, 1:1. and 2:2) per µg of DNA. Transfections were performed in 24-well deep well shaker blocks using FreeStyle™ CHO-S cells cultured in BD Select™ CD1000 media (2 ml/well). Cells were plated at a density of 0.5 x 10^6 cells/mL at the time of transfection, harvested 48 hours post-transfection and assayed using a conventional luciferase assay. Error bars represent the standard deviation of triplicate wells.

Media Compatibility



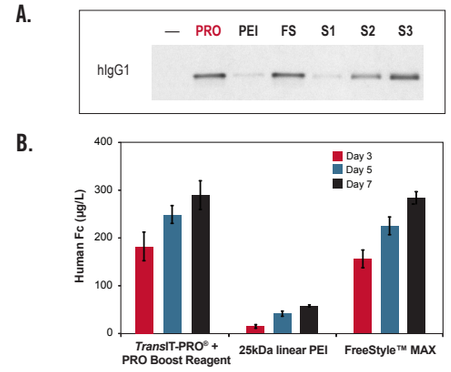
***Trans*IT-PRO® provides high performance across varied media formulations.** FreeStyle™ CHO-S cells were adapted to five representative growth media including: BD Select™ CD1000 medium (Becton, Dickinson and Company, Franklin Lakes, NJ), BD Select™ CHO medium (Becton, Dickinson and Company, Franklin 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 using the *Trans*IT-PRO and PRO Boost Reagent (1:0.5:1), 25kDa linear PEI (6:1) (Polysciences, Warrington, PA), or FreeStyle Max (1:1) (Life Technologies Corporation, Carlsbad, CA) transfection reagents according to the published protocol (reagent:DNA ratio). Transfections were performed in 24-well deep well shaker blocks using 1 µg plasmid DNA per milliliter of culture and 0.5 x 10^6 cells/ml at the time of transfection. (A) Luciferase expression was assessed 48 hours post-transfection using a conventional luciferase assay. Error bars represent the standard deviation of triplicate wells. (B) Human IgG1 was quantitated from day 5 clarified supernatants and analyzed by a human anti-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate wells.

Scalability



Scaling of transient transfection using *Trans*IT-PRO® Transfection Kit is linear from 4 to 400 milliliters. Human IgG1 was produced by transient transfection using the *Trans*IT-PRO Transfection Kit and a plasmid encoding a human IgG1 construct. A DNA concentration was 1µg/ml of culture and a ratio of *Trans*IT-PRO:PRO Boost Reagent:DNA ratio of 1:0.5:1. Cells were plated at a density of 0.5 x 10^6 cells/ml at the time of transfection. CHO-S cells were cultured in BD Select™ CD1000 media using 4 ml per well of a 24-well deep well shake block, 40 ml in 125 ml Erlenmeyer shake flask, 40 ml in 125 ml 2 sidearm spinner flask and 400 ml in 500 ml 2 sidearm spinner flask . Day 3, 5 and 7 supernatants were clarified and analyzed by an anti-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate technical replicates.

Competitor Benchmarking



Achieve high antibody titers using the *Trans*IT-PRO® Transfection Kit. Human IgG1 was produced by transient transfection using *Trans*IT-PRO and PRO Boost Reagent (1:1:1), 25kDa linear PEI (6:1) or FreeStyle™ Max (1:1) 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 10^6 cells/mL at the time of transfection. FreeStyle™ CHO-S cells were cultured in 20mL of FreeStyle™ CHO Expression medium in 125 ml shake flasks. (A) Day 7 supernatants were clarified and analyzed by Western blot. An IgG standard was included for quantification estimate (S1= 1.6 mg/L, S2= 3.2 mg/L, S3 = 6.3 mg/L) (B) Day 3, 5 and 7 supernatants were clarified and analyzed using a human IgG-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate technical replicates, 25kDa linear PEI is duplicate technical replicates.

Conclusions

- Achieve high protein yield in suspension CHO cells
- Easy to Use - No media change required
- Minimal optimization results in high protein expression
- Compatible with multiple CHO media formulations
- Scalable from 4 - 400 milliliters of culture volume