



The Transfection Experts

Selecting a Transfection Reagent for Large Scale Protein Production in Suspension 293 Cell Types

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Suspension cell lines derived from HEK 293 (Human Embryonic Kidney) and CHO (Chinese Hamster Ovary) cells are commonly used in the production of pharmaceutical biotherapeutics. These cell lines have many desirable traits including high expression levels, scalability (density and volume), and a history of regulatory approval. Clinical biotherapeutics are frequently generated using stable high-expression transfectants for batch-to-batch consistency and low cost at extremely large-scale; however, many advances in the last decade including improved cell lines, expression vectors, culture medium, and delivery methods have led to the adoption of transient transfection for mammalian protein expression. In many drug discovery applications, it is beneficial to screen protein constructs quickly using transient transfection methods (< 1 week); allowing for the evaluation of various target molecules or protein isoforms simultaneously. In many instances, transient transfections are performed in parallel while more resource-intensive stable cell lines are under development (> 3 months).

One of the primary challenges in biopharmaceutical manufacturing is the design of cost-effective systems for large-scale protein production. Several parameters influence the protein yield in transient transfections including the cell type, transfection reagent, protein, medium formulation, etc. HEK 293 derived suspension cells are commonly used in early development stages since they routinely produce high protein titers. At later stages, suspension CHO cell transient transfection and stable cell line generation often leads to the final biotherapeutic.

A key step in establishing an efficient system is pairing a cell type suitable for transfection with a high efficiency transfection reagent. Recent advances in transfection technologies, which include the *TransIT-PRO*[®] Transfection Kit by Mirus Bio, have allowed researchers to obtain high protein titers in suspension 293 and CHO derived cells in a simple and reproducible manner. With regards to Mirus Bio's technology, transfection complexes are prepared in serum-free media by adding plasmid DNA, *TransIT-PRO* Transfection Reagent, and PRO Boost Reagent. The PRO Boost Reagent is an optional component and enhances gene expression in certain growth media formulations. After incubating complexes for 10-30 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. *TransIT-PRO* performs similarly or better than 293fectin[™] using the FreeStyle[™] 293 Expression Medium. Ten secreted (non-antibody) proteins were expressed at a scale of 1-6L using both *TransIT-PRO* and 293fectin Transfection Reagents (Figure 1).

Cost of the transfection reagent is a barrier to researchers and many seek low cost alternatives such as linear 25 kDa Polyethyleneimine (PEI). Due to the nature of the polymer, PEI is difficult to solubilize, prone to high batch-to-batch variability and low transfection efficiencies. In commonly used 293 and CHO suspension cell lines, we routinely observe 3-5 fold lower protein expression with PEI compared to *TransIT-PRO* Transfection Reagent. Therefore, the increase in materials and labor required to produce the same amount of protein more than offset the cost of the transfection reagent. Figure 2 outlines a hypothetical cost breakdown where a researcher desires 10 mg of a protein. With this particular

construct, sufficient amounts of protein can be obtained with a 1 L culture using the *TransIT-PRO* Transfection Reagent; however, due to lower protein expression, 3 L of culture are required for PEI-mediated transfection. The labor is multiplied by a factor of 1.5 to account for the increase in handling and purification time with the increased volume. Based on this scenario, the total cost of the PEI-mediated transfection is approximately one-third higher than the *TransIT-PRO* Transfection (\$1,886 vs \$1,367.50) even though the reagent is much less expensive. Additionally, significant cost savings with the transfection reagent can often be realized if it is purchased in bulk quantities.

Protein yield is highly dependent on the intrinsic properties of the recombinant protein; two antibody constructs of the same subtype can produce vastly different antibody titers. Protein titers span a broad range although secreted antibodies, especially the IgG1 subclass, tend to be higher. This trend is depicted in Figure 3, where 8 different antibodies and 21 different secreted proteins were transiently expressed in 293 suspension cells using similar experimental conditions. The protein yields range from 1.1 - 49.3 mg/L depending on the protein.

Media formulation also has a large impact on transfection efficiencies. The complex formation media is critical for proper condensation of the nucleic acid which influences the size and charge of the transfection complexes. We recommend OptiPRO™ SFM (Life Technologies), because of its animal origin free nature. In addition, the cellular growth media can influence transfection efficiencies. This is likely due to the complex nature of the serum-free complete media formulations that support high density cell growth. It is important to test several media formulations to ensure that high transient transfection efficiencies are obtained. In general, media growth supplements can be added 18-24 hours post-transfection without any adverse effects on transfection efficiencies.

High efficiency transient transfection of suspension HEK293 derived cells enables efficient manufacture of complex biotherapeutics. Herein, we have demonstrated that the *TransIT-PRO*® Transfection Kit provides high efficiency transfection of suspension 293 cells thereby saving valuable time and lowering research costs.

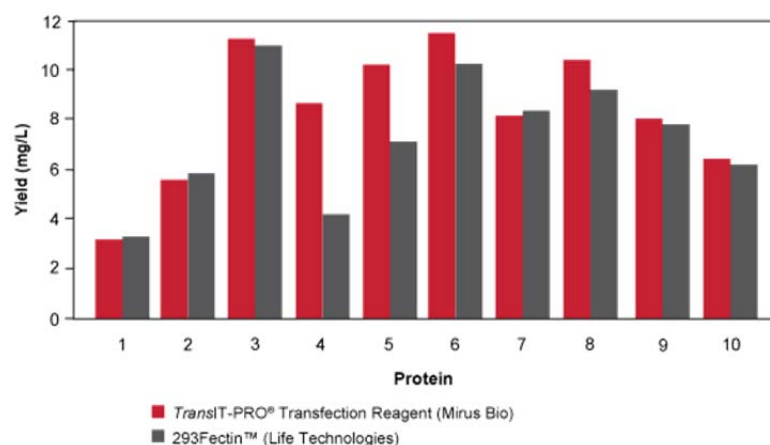


Figure 1. Achieve High Protein Yields Using the *TransIT-PRO*® Transfection Kit in Suspension 293 Cells. Ten different secreted (non-antibody) proteins were transiently expressed in FreeStyle™ 293-F cells (Life Technologies) using the *TransIT-PRO*® (1.5:1) and 293fectin™ (Life Technologies, 2:1) transfection reagents according to manufacturers protocol. Cells were grown in FreeStyle™ 293 Expression Medium at transfected at a density of 1,000,000 cells/ml. The scale of the transfection for each protein varied between 1-6 L of culture.

Data courtesy of a *TransIT-PRO*® pharmaceutical customer.

Hypothetical Scenario:
Materials needed for 1L Transfection, 10mg desired yield

	<i>TransIT-PRO</i> [®]	PEI
		materials 3x, labor 1.5x
1L media	75.00	225.00
100 mL OptiPRO [™] Formation Media	18.50	55.50
1.0 mg DNA	100.00	300.00
Transfection Reagent		
<i>TransIT-PRO</i> [®] (1:1 ratio)	364.00	
25 kDa Linear PEI		0.84
Disposable 1L Culture Flask	60.00	180.00
Time in hours * \$150 per hour	750.00	1,125.00
TOTAL	\$1,367.50	\$1,886.34

Figure 2. Estimated time and material comparison between transient transfections performed using *TransIT-PRO*[®] and 25 kDa linear PEI. Hypothetical scenario considers that a researcher desires 10 milligrams of protein which can be obtained from a 1L culture of 293 suspension cells transfected with the *TransIT-PRO*[®] Transfection Reagent. Due to 3-5 fold lower yields with PEI, the material costs are multiplied by a factor of 3 and labor hours by 1.5 to account for the disparity in transfection efficiencies to obtain the 10 milligrams of protein. Cost of materials and labor is estimated based on list price and/or fair market value.

Protein Type	Scale (L)	Yield (mg/L)
Antibody	1	18.1
	5	15.4
	6	25.0
	6	11.2
	6	20.0
	5	18.8
	10	13.3
	11	33.3
Other Secreted Proteins	1	7.4
	1	5.3
	1	10.3
	1	3.9
	1	5.5
	1	17.9
	1	8.4
	1	16.3
	1	7.5
	1	17.2
	1	49.3
	1	10.7
	1	3.1
	1	2.0
	1	1.7
	1	24.9
	1	1.1
	1	1.1
1	16.5	
1	24.5	
1	28.2	

Figure 3. Yield is Dependent on the Intrinsic Properties of the Protein. Eight different antibodies and 21 different secreted proteins were transiently expressed in FreeStyle[™] 293-F cells (Life Technologies) using the *TransIT-PRO*[®] Transfection Reagent (1:1) according to manufacturer’s instructions. Cells were grown in FreeStyle[™] 293 Expression Medium and transfected at a density of 1,000,000 cells/ml. The scale of the transfection for each protein varied between 1-11 L of culture. The protein yields range from 1.1 - 49.3 mg/L

Data courtesy of a *TransIT-PRO*[®] CRO customer.

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