Corning® CellSTACK® Culture Chamber PEIpro® Transfection Protocol

Guidelines for Use

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This document provides a generalized procedure for chemical-based DNA transfection using PEIpro transfection reagent in Corning CellSTACK culture chambers. PEIpro transfection reagent (Polyplus Cat. No. 115-100) is a leading chemically defined linear polyethylenimine optimized for large-scale virus production in common mammalian producer cell lines[1]. The transfection protocol described is intended as a starting point for those wishing to transfect large numbers of cells in the Corning CellSTACK-10 chamber (Corning Cat. Nos. 3270 or 3271), especially for viral production.

As a simple proof-of-concept, the protocol was developed for HEK293T cells using a high-expression green fluorescent protein (GFP) control plasmid, gWIZ™-GFP (Aldevron Cat. Nos. 5006 or 5007). In this instance, GFP provided a convenient reporter for both visual inspection of the transfected chambers and assay of transfection efficiency using the NucleoCounter® NC-3000™ Advanced Image Cytometer[2]. The procedure discussed can be adapted for other cell types and to accommodate co-transfection of multiple plasmids for viral vector production. In addition, transfections using PEIpro transfection reagent can be easily scaled for process optimization in any size Corning CellSTACK culture chamber prior to large-scale transfections in multiple CellSTACK-10 chambers.

Cell Seeding

Follow standard culturing protocols to establish HEK293T cultures (ATCC® Cat. No. CRL-3216™) in Dulbecco's Modified Eagle Medium (DMEM, Corning Cat. No. 10-013-CM) plus 10% fetal bovine serum (FBS, Corning Cat. No. 35-010-CV). Forty-eight to seventy-two hours prior to transfection (Day 0), plate cells at 5 x 10^3 cells/cm^2 into the desired number of CellSTACK-10 chambers to be transfected (636 cm^2 each; Table 1). Seed one additional CellSTACK-10 chamber to harvest on the day of transfection for cell density determination. Incubate at 37°C in 5% CO_2 until cultures reach approximately 40% to 60% confluence or 7 x 10^4 to 9 x 10^4 cells/cm^2 (Days 2 through 3; Figure 1).

NOTE: Refer to the Corning CellSTACK Culture Chambers Instructions for Use (CLS-BP-007DOC) for handling of the CellSTACK-10 chamber[3].

NOTE: For troubleshooting, see Helpful Hints (Sections 1 through 3) at the end of this document.

Table 1. Sample Volumes for Scaling Corning CellSTACK Chamber Transfections

Vessel	Area (cm^2)	Cell Density at Time of Transfection (cells/cm^2)	Total Cell Number (cells)	Volume Serum-free Medium (mL)	DNA Quantity (mg)	Volume PEIpro Reagent (mL)
Corning CellSTACK-1 chamber	636	8 x 10^4	5.1 x 10^7	7.5 + 7.5	0.1	0.2
Corning CellSTACK-2 chamber	1,272	8 x 10^4	1.0 x 10^8	15 + 15	0.2	0.4
Corning CellSTACK-5 chamber	3,180	8 x 10^4	2.5 x 10^8	38 + 38	0.5	1.0
Corning CellSTACK-10 chamber	6,360	8 x 10^4	5.1 x 10^8	75 + 75	1.0	2.0
Corning CellSTACK-40 chamber	25,440	8 x 10^4	2.0 x 10^9	300 + 300	4.1	8.2

Preparation of Complexes

1. On the day of transfection (Days 2 through 3), harvest cells from one of the CellSTACK-10 chambers with Trypsin EDTA (Corning Cat. No. 25-052-CV) plus 0.1% Poloxamer 188 (Corning Cat. No. 13-901-CI) for 10 minutes at room temperature following 1X Ca^{2+}/Mg^{2+}-free Dulbecco's Phosphate-Buffered Saline (DPBS, Corning Cat. No. 21-031-CM) wash.

   NOTE: Incorporating 0.1% Poloxamer 188 into the harvest solution can enhance cell recovery and reduce cell clumping.

   NOTE: For detailed information on basic cell harvesting procedures in the CellSTACK-10 chamber, refer to Corning Protocol CLS-AN-076[4].

2. Prepare three 1 mL samples from the harvested cell suspension for enumeration.

3. Measure cell count and viability for each sample using the Via1-Cassette™ (ChemoMetec Cat. No. 941-0012) and NucleoCounter NC-3000 (ChemoMetec) according to manufacturer's instructions. Calculate total cell number per chamber as an average of the 3 sample measurements.

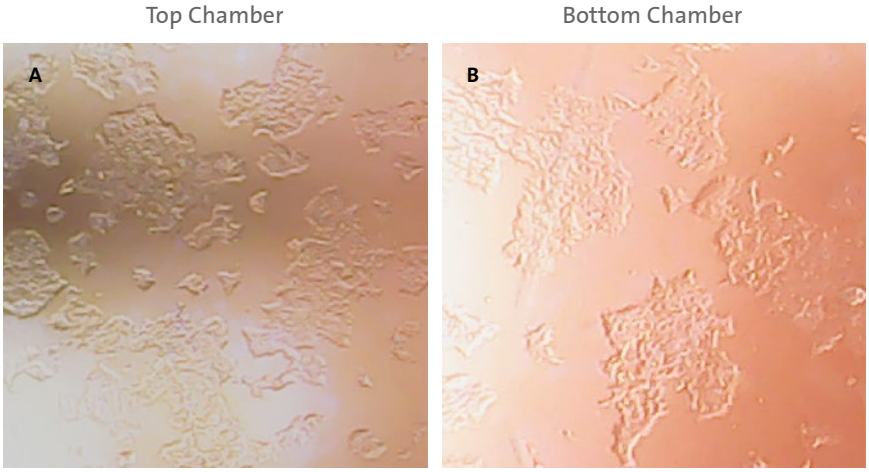

Figure 1. Confluence on the day of transfection. Representative images of HEK293T in the top (A) and bottom chamber (B) of the Corning CellSTACK-10 chamber on the day of transfection. Images were acquired with a handheld USB microscope (Bysameyee Microscope 1000X).

4. Calculate the total amount of plasmid DNA to add per chamber as follows:

$$\text{Total µg DNA} = \frac{\text{Total cell number per chamber}}{1 \times 10^6} \times (\text{µg DNA per } 1 \times 10^6 \text{ cells})$$

NOTE: Recommended DNA amounts range from 1 µg to 2 µg per 1 x 10^6 cells, whether it is a single plasmid or multiple plasmids for co-transfection. Each plasmid should represent at least 10% of total DNA. The total amount of DNA should be determined empirically for the desired application with preliminary studies to optimize for high efficiency transfection. Calculations in Table 1 are based on 2 µg per 1 x 10^6 cells.

NOTE: For troubleshooting, see Helpful Hints (Sections 4 through 6) at the end of this document.

5. Calculate the amount of PEIpro® reagent as follows:

Volume PEIpro reagent = (Total µg DNA) x (µL PEIpro per µg DNA)

NOTE: Recommended PEIpro reagent concentration ranges from 1 µL to 2 µL per µg of DNA. The PEIpro reagent to DNA ratio should be determined empirically for the desired application with preliminary studies to optimize for high efficiency transfection. Calculations in Table 1 are based on 2 µL PEIpro reagent per µg of DNA. For specific instructions on the use of PEIpro, contact Polyplus Technical Support[1].

NOTE: For troubleshooting, see Helpful Hints (Sections 4 through 6) at the end of this document.

6. For each CellSTACK-10 chamber to be transfected, pipette 75 mL DMEM into each of 2 sterile 150 mL storage bottles (Corning Cat. No. 431175).

NOTE: DNA and PEIpro reagent are diluted in equal volumes of serum-free medium for a total volume of transfection complexes that is 10% of the total CellSTACK chamber volume. Scale accordingly for each size of CellSTACK chamber or for transfection of multiple chambers. Calculations in Table 1 are based on a working volume of 150 mL per chamber.

7. Add the DNA to one bottle of DMEM. Vortex gently.
8. Add the PEIpro® reagent to the second bottle of DMEM. Vortex gently.
9. Add the PEIpro solution to the DNA solution prepared in Step 7. Vortex immediately.
10. Incubate the complexes for 15 minutes at room temperature.
11. The transfection complexes are now ready for addition to the CellSTACK-10 chamber.

Transfection in the Corning® CellSTACK® Culture Chamber

1. Remove the CellSTACK-10 chamber from the incubator. Pool medium into the lowest layers by tilting the vessel on a port corner to allow as much medium as possible to pool into the lowest layers.

NOTE: This is done to facilitate mixing of the complexes within the CellSTACK chamber (Figure 2A).

2. Using a 100 mL pipet, pipette all transfection mix prepared above to the lowest layer of the CellSTACK-10 chamber (Figure 2B).
3. Follow the procedure for medium equilibration in the Corning CellSTACK Culture Chambers Instructions for Use (CLS-BP-007DOC) to equilibrate medium throughout the layers of the unit[3]. Briefly, set the chamber on its side (Figure 2C) to allow medium to level out between all layers, rotate the chamber 90° so that the filling and venting ports are up, and then gently lower the CellSTACK chamber to its normal horizontal incubation position[3].

4. Repeat Steps 1 and 3 twice more.
5. Incubate at 37°C, 5% CO_2 until desired harvest.

   NOTE: For troubleshooting, see Helpful Hints (Sections 6 and 7) at the end of this document.

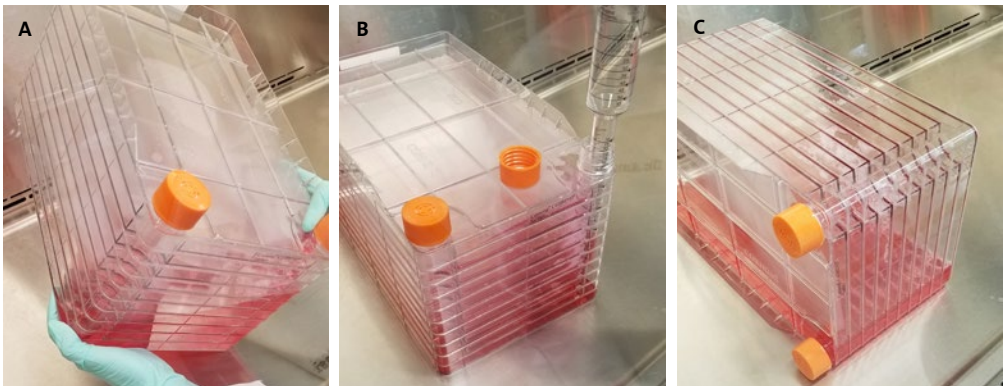

Figure 2. Mixing of transfection complexes in the Corning CellSTACK-10 chamber. Medium is pooled in the lowest layers of the CellSTACK-10 chamber (A) before addition of the transfection mix (B). Medium equilibration (C) followed by additional medium pooling and equilibration steps facilitates mixing of the transfection complexes within the CellSTACK chamber.

GFP Transfection Efficiency Assay

The following section details the protocol to determine transfection efficiency for cultures that have been transfected with a GFP reporter gene. If cells have been transfected with another reporter or plasmid(s) without a reporter construct, process the transfected cells as necessary.

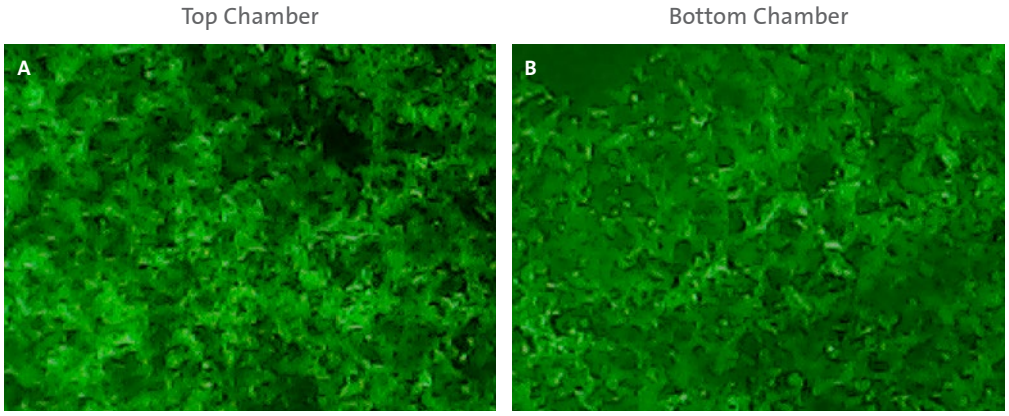

Figure 3. GFP fluorescence at 48 hours post-transfection. Representative images of GFP fluorescence from the top (A) and bottom (B) chamber of a Corning CellSTACK 10-layer chamber at 48 hours post-transfection. GFP was excited *in situ* with a blue (440 to 460 nm) LED flashlight to illuminate the top layer of the CellSTACK-10 chamber. Fluorescence was filtered with a yellow barrier filter for image acquisition a handheld USB microscope.

1. Harvest cells from the transfected CellSTACK-10 chamber and enumerate the cell suspension as in Step 1 of "Preparation of Complexes" section.
2. Prepare 3 or more samples from the harvested cells, diluting as necessary with DPBS to a final concentration of 5 x 10^4 cells/mL to 5 x 10^6 cells/mL.
3. Pipette 96 µL of each cell suspension into a microcentrifuge tube (Corning Cat. No. 3620 or 3621).
4. Add 2 µL of Solution 15 (Hoechst-33342; ChemoMetec Cat. No. 910-3015) and mix by pipetting. Incubate in a dry bath heater (Corning Cat. No. 6875-SB) at 37°C for 15 minutes.
5. After incubation, add 2 µL of Solution 16 (Propidium Iodide; ChemoMetec Cat. No. 910-3016) and mix by pipetting.
6. Load approximately 30 µL of the stained cell suspension into each chamber of an NC-Slide A2 (ChemoMetec Cat. No. 942-0001) and run the "GFP-Transfection Assay-Hoechst and PI" on the NucleoCounter® NC-3000™ according to manufacturer's instructions. Store additional loaded slides in the dark until they are processed.
7. Following image acquisition, view summary data of transfection ratios for viable and nonviable cells in the Results tab. To more accurately determine transfection ratios, detailed analysis of histograms and gating of scatter plots is recommended after data acquisition, and can be accessed in the Plot Manager window. For specific instructions, contact ChemoMetec Technical Support[2].

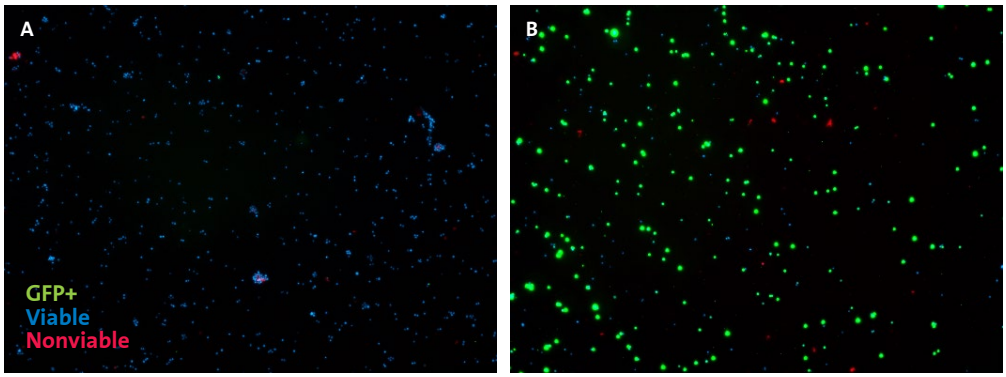

Figure 4. NucleoCounter NC-3000-acquired Images for transfection efficiency analysis. Representative cytometry images from control cells transfected with PEIpro reagent alone (A) and GFP transfected cells (B). Green cells are GFP-expressing cells, red cells are nonviable cells, blue cells are GFP-negative viable cells.

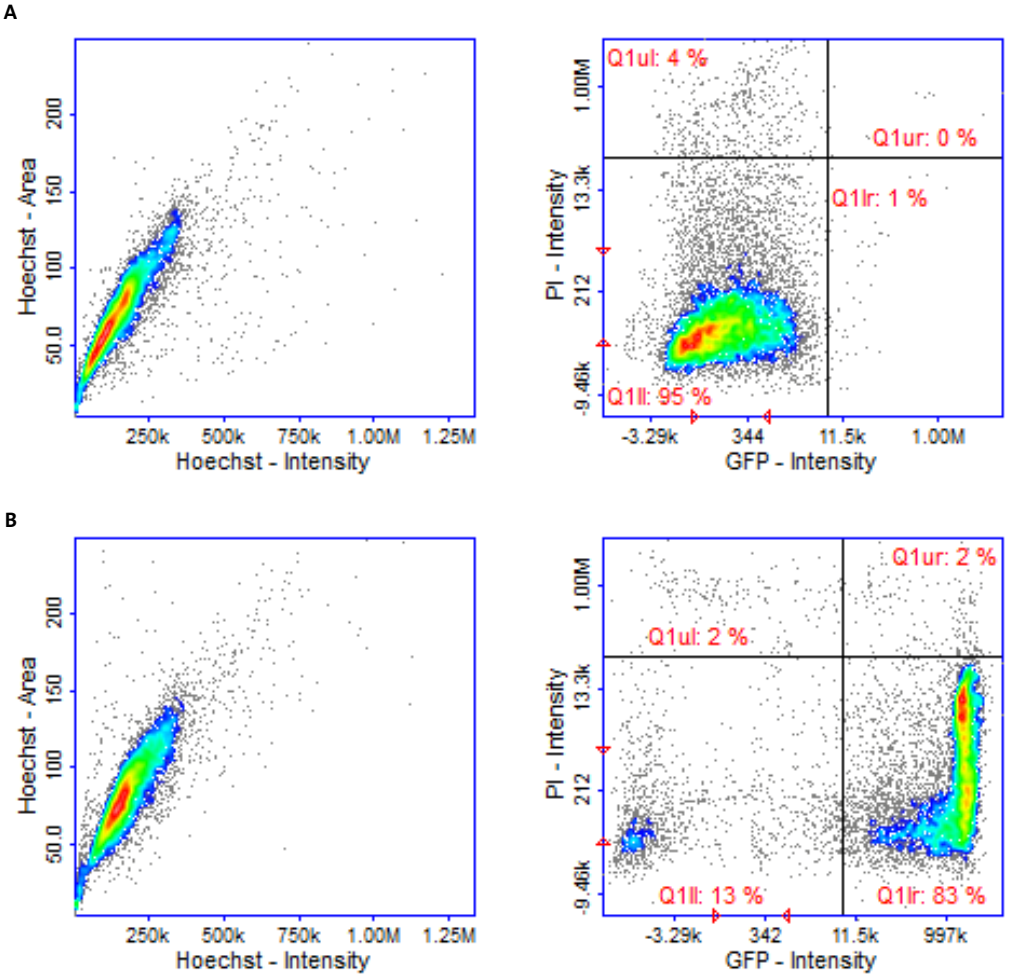

Figure 5. Scatter plots for gating of GFP-positive cell population. Representative scatter plots using NucleoView™ software (ChemoMetec) corresponding to the cytometry images in Figure 4 from control cells transfected with PEIpro reagent alone (A) and GFP transfected cells (B). Hoechst scatter plot (left) shows distribution of the live population of cells. GFP gating and analysis (right) in a scatter plot of GFP intensity versus PI intensity. The GFP-negative control cell population was used to set gates for GFP expression in this study. The PI intensity was used to set gates for viable/nonviable cells.

Helpful Hints

1. Transfection efficiency is highly dependent upon cell health. Maintain cultures below 80% to 90% confluence with regular subculture, and conduct transfections on early passage cultures. **NOTE:** Do not allow cells to become overconfluent before subculture, as contact inhibition will slow culture growth and thus, negatively impact transfection efficiency.
2. The protocol outlined above was optimized to favor a longer time course, ensuring full recovery from subculture for high viability and active cell division at the time of transfection. Seeding density and time in culture prior to transfection can be adapted to fit alternative timelines for different cell types or applications, provided cells are actively dividing at the time of transfection.
3. If cells become detached following transfection as a product of protein or viral vector expression or due to toxicity, seed cells on Corning® CellBIND® CellSTACK® vessels to enhance cell attachment during transfection.
4. Use only high quality plasmid preparations that are free of proteins, RNA, and endotoxins.
5. Optimize the PEIpro® reagent to DNA ratio and the amount of plasmid DNA to increase transfection efficiency.
6. In some instances, cellular toxicity may be observed following transfection. If toxicity is observed, decrease the PEIpro reagent to DNA ratio or the amount of plasmid DNA, which is especially important if the expressed protein is toxic. In addition, it may be necessary to change culture medium 4 to 6 hours post-transfection.
7. PEIpro reagent is suitable for transfections in the presence of serum and antibiotics. If using a serum-free medium, ensure that the medium is permissive to transfection.

References

1. Polyplus Transfections Technical Support at support@polyplus-transfection.com
2. ChemoMetec Technical Support at https://ChemoMetec.com/contact/science-support-center/
3. Corning CellSTACK Culture Chambers Instructions for Use (CLS-BP-007DOC).
4. Harvesting Cells from Corning CellSTACK Culture Chambers Protocol (CLS-AN-076).

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