SARTORIUS

Simplifying Progress

Addressing Large-Scale Therapeutic Virus Production Using High Quality Grade PEI-Based Transfection Reagents

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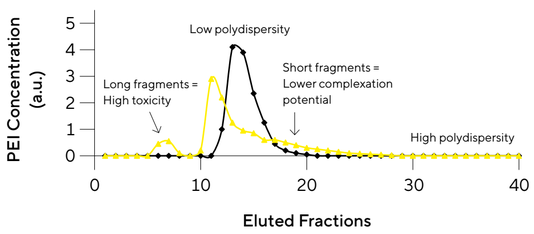
1. Introduction

Gene and cell therapy-based medicines are experiencing resurgence due to the introduction of "next generation" transfer viral vectors, which have demonstrated improved safety and efficacy. Adeno Associated Virus (AAV) and Lentivirus are very commonly used in therapeutics and often produced using PEI-mediated transient transfection in HEK-293 or HEK-293T cells. The critical raw materials needed for cGMP vector production must be sourced from approved suppliers and should have gone through a rigorous testing program to reduce the risk of introducing adventitious agents into the production process. Polyplus-transfection now provides PEIpro®, the unique PEI-based transfection reagents available in different quality grades, allowing a seamless transition from process development with PEIpro®-HQ to cGMP biomanufacturing with PEIpro®-GMP. Here, we describe an optimized PEI-based virus production process for high-yielding viral vector production, compatible with different cell culture adherent and suspension systems. We further demonstrate the robust viral vector production yields, as well as the adaptability and reliability of the PEI-based transient gene expression approach to efficiently manufacture GMP-grade viral vectors at a sufficiently large scale for more advanced clinical trials, and in fine to drive commercialization of therapeutic vectors.

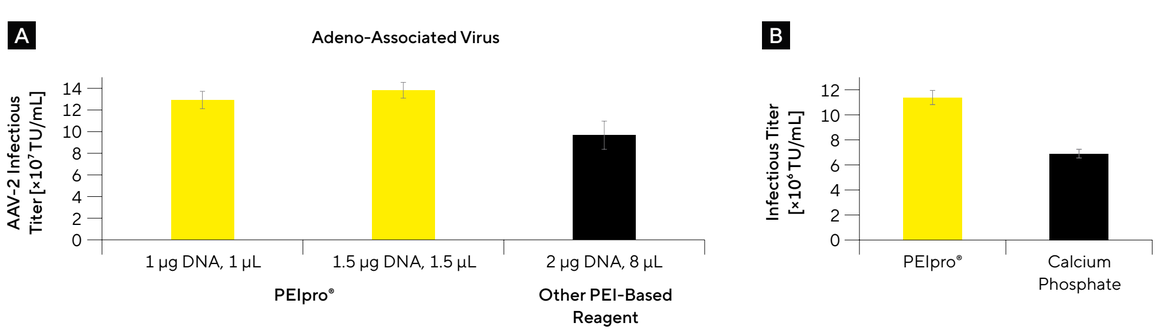
2. Optimized Transient Transfection for Virus Production

Figure 1: *Optimization Process of PEI Polymer Chemistry*



Note. Whereas long polymer fragments lead to cell toxicity and short fragments lead to lower complexation potential (in yellow), optimized PEI size with a low polydispersity index decreases toxicity and increases complexation potential (in black) and reproducibility in transfection.

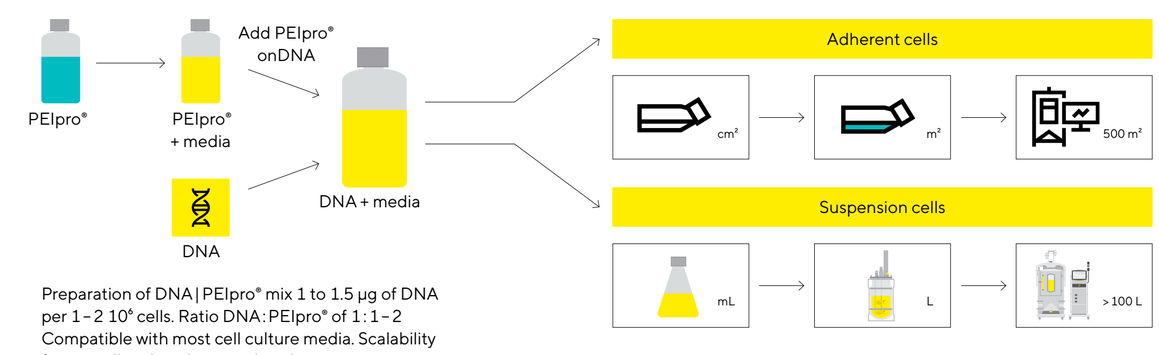
Figure 2: *PEIpro® Produces More Virus With Less Reagent and Lower DNA Amount Compared to Another PEI-Based Reagent and Calcium Phosphate Transfection*



Note. **(A)** Suspension HEK-293T cells were seeded at 1 × 10⁶ cells/mL in serum-free medium and transfected with PEIpro® and another PEI-based reagent following the recommended protocols. AAV-2 were produced with Helper Free Packaging System (Cell Biolabs, San Diego, CA) and titers were measured 72 hr after transfection using a GFP reporter gene expression. **(B)** Lentiviruses were produced in adherent HEK-293 cells grown in serum-free culture medium, using 15 µg DNA and 30 µl PEIpro® per 75 cm² flask. Virus yields were determined by titration of the supernatant 48 hr after transfection.

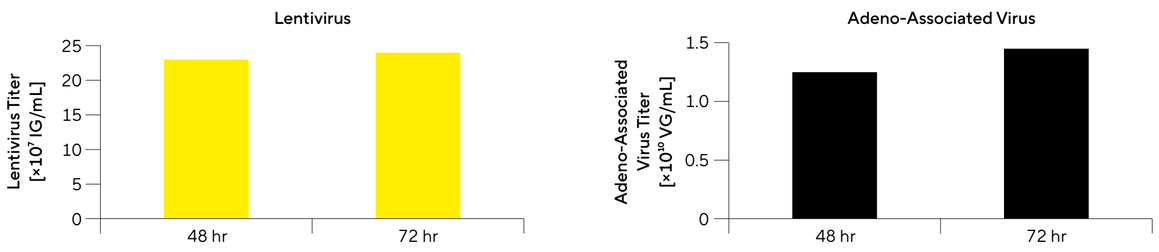
3. Efficient Virus Production in Any System at Any Scale

Figure 3: *PEIpro® Is the Reagent of Choice for Virus Production Runs in Most Cell Culture Systems in Both Adherent and Suspension Cells, From Small Scale to Large Scale*



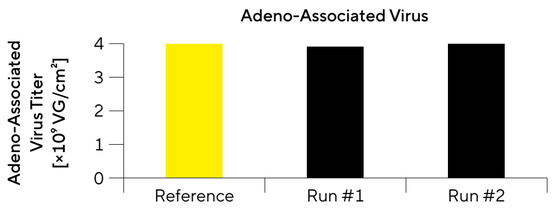
Preparation of DNA | PEIpro® mix 1 to 1.5 µg of DNA per 1 – 2 10⁶ cells. Ratio DNA : PEIpro® of 1 : 1 – 2
Compatible with most cell culture media. Scalability from small scale to large scale volume

Figure 4: *Lentivirus and AAV Production in HEK-293T and HEK-293 Cells Grown in Suspension in BalanCD® HEK-293 (Irvine Scientific®)*



Note. HEK-293T (lentivirus) and HEK-293 (AAV) cells were thawed directly into each medium and passaged every 3 to 4 days before going into a 2 L benchtop bioreactor. Cells were seeded and cultured for 3 days before being transfected with PEIpro® (Polyplustransfection®). For transfection, four plasmids were used for lentivirus and three plasmids were used for AAV. Lentiviral and AAV titer were measured 48 and 72 hours post-transfection (Data kindly provided by Généthon).

Figure 5: *PEIpro® to Simplify Scale-Up and to Ensure Reproducible Virus Production Yields in iCELLis® Nano Bioreactor*



Note. AAV-8 production in iCELLis® Nano 0.8 m² and 4 m². Triple PEIpro®-mediated transfection in Freestyle™ F17 medium using 1.0 µg DNA/million cells and medium exchange with DMEM applied 5 hr post-transfection. Data are based on in situ cell lysis and AAV recovery at 72 hr posttransfection. qPCR analysis was performed on cell lysate (Data kindly provided by Pall).

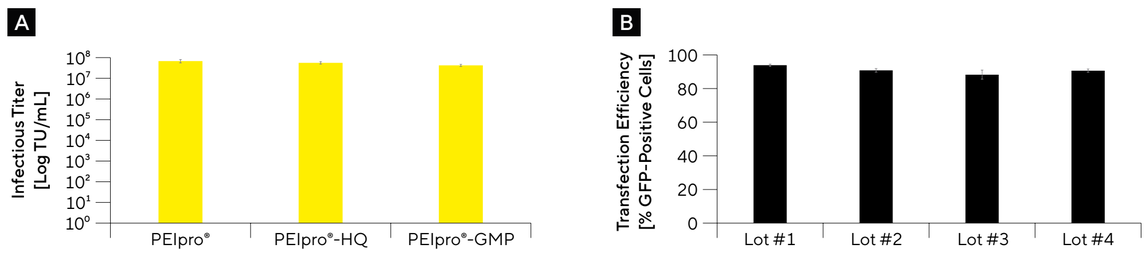
4. Seamless Transition From Process Development up to Clinical Trials and Commercialization

Table 1: *Range Of PEIpro® Quality Grade Rreagents for Each Step Of Nucleic Acid-Mediated Viral Vector-Based Manufacturing*

Characteristics	PEIpro®	PEIpro®-HQ	PEIpro®-GMP
	Process Development	**Pre-Clinical and Early Phase Clinical Trial**	**Clinical Trials and Commercialization**
Quality Grade	R&D grade	Pre-clinical grade	GMP grade
Composition	Ready to use, chemically defined and animal derived component free		
Packaging	Bottles	Bottles	Bottles Bags (closed system)
Available pack size	1.5 mL bottle 10 mL bottle 100 mL bottle 1 L bottle	100 mL bottle 1 L bottle	10 × 10 mL bottles 100 mL bottle 300 mL bag 1 L bag
Fill & finish manufacturing process	Sterile filtration	Sterile filtration	Sterile filtration Validated aseptic process
Quality controls	Standard QCs	Extended QCs to assess identity, potency, purity and safety	Validated QCs according to European Pharmacopeia assessing identity, potency, purity and safety
Included documentation	▪ Certificate of analysis ▪ Certificate of origin ▪ Non-hazardous product statement	▪ Certificate of analysis ▪ Certificate of origin ▪ Non-hazardous product statement	▪ Certificate of analysis ▪ Certificate of compliance ▪ Certificate of origin ▪ Non-hazardous product statement
Regulatory documentation available upon request		▪ Batch production documentation ▪ Quality agreement	▪ DMF (drug master file) on file (FDA) for USA ▪ CMC section (chemistry, manufacturing and control) for the rest of the world ▪ Protocol for identity testing ▪ Quality agreement
Audit	According to ISO 9001 2015	According to ISO 9001 2015	According to ICH Q7, GMP Part II and Annex I

Note. PEIpro® is available as an R&D grade for establishment of viral vector production during process development. For production of clinical batches of viral vectors, we supply higher preclinical grade PEIpro®-HQ and highest quality grade PEIpro®-GMP to meet the quality demands of both cell therapy and gene therapy.

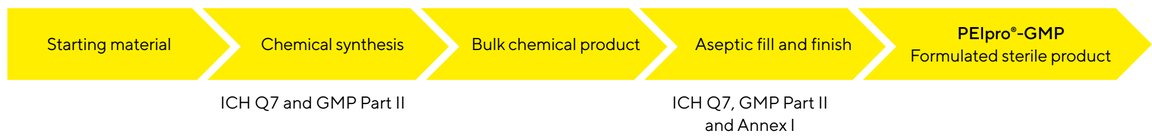
Figure 6: *High Reproducibility With PEIpro® Regardless of the Quality Grade and the Production Lot*



Note. **(A)** Suspension HEK-293T cells were seeded at 1 × 10⁶ cells/mL in FreeStyle™ F17 medium and transfected with either PEIpro®, PEIpro®-HQ or PEIpro®-GMP reagents following the same protocol for each product. AAV-2 were produced with Helper Free Packaging System (Cell Biolabs, San Diego, CA) and titers were measured 72 hr after transfection using a GFP reporter gene expression. **(B)** Suspension HEK-293T cells were seeded at 1 × 10⁶ cells/mL in FreeStyle™ F17 medium and transfected with four different lot of PEIpro®-GMP with a GFP-expressing plasmid. Transfection efficiency was measured 48 hours post-transfection by flow cytometry.

5. PEIpro®-GMP: Highest Quality Grade PEI Available

Figure 7: *Manufacturing Process of PEIpro®-GMP*



Note. PEIpro®-GMP is manufactured according to a validated manufacturing process in compliance with GMP guidelines to ensure traceability from starting material to the final product. GMP guidelines for manufacturing of ATMP requires that raw materials be of pharmaceutical grade when available (ICH Q7 and Eudralex Vol 4, Part II, Annex I). To address this requirement, both steps of PEIpro®-GMP manufacturing (chemical product and fill & finish) are managed in compliance with GMP guidelines in GMP accredited facilities.

6. Conclusion: Advantages of PEIpro® Product Range

- Best-in-class PEI-based transfection reagent for viral vector production
- Seamless transition from process development up to clinical trials and commercialization
- Higher quality grade PEIpro®-HQ and PEIpro®-GMP to meet compliance requirements
- Chemically defined and animal derived component free