Addressing Large-Scale Manufacturing of Clinical Grade Viral Vectors Using an Optimized PEI-based Transfection Process

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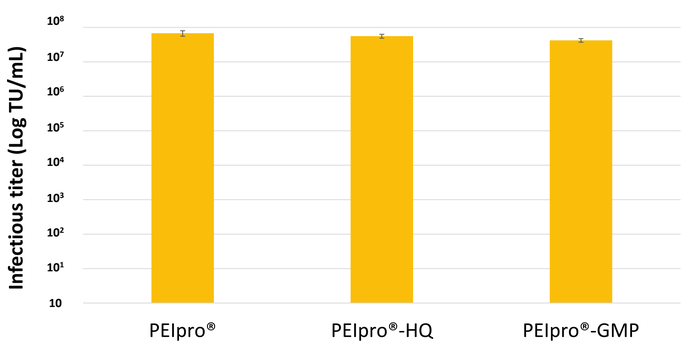
Abstract

With the progress in developing new viral vector systems guided by safety, specificity and potency considerations, several gene and cell-based therapies are now more than ever closer to being clinically approved and commercially available to treat genetic diseases.
Viral vector delivery systems, of which mainly adeno-associated viruses (AAV) and lentiviruses are produced by transient transfection of mammalian producer HEK-293 cell lines. Virus vector production using the right transient transfection method is crucial to provide the flexibility and reproducibility that is needed to scale-up from initial process development to manufacturing of high-quality grade viral vectors.
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.

Seamless transition from process development up to clinical trials and commercialization

Characteristics	PEIpro®	PEIpro®-HQ	PEIpro®-GMP
	Process development	Pre-clinical & early phase clinical trial	Clinical trials & 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	Bags (closed system)
Available pack size	1.5 mL 10 mL 4 x 10 mL 100 mL 4 x 100 mL	4 x 100 mL 10 x 100 mL 1 L	1L
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 - TSE/BSE Statement - Non-Hazardous Product Statement
Regulatory Documentation available upon request		- Batch Production Documentation - Quality agreement	- DMF (Drug Master File) on file (FDA) - CMC section (Chemistry, Manufacturing and Control) - Protocol for incoming testing - Quality agreement
Audit	According to ISO 9001 2015	According to ISO 9001 2015	According to ICH Q7, GMP Part II and Annex I

Range of PEIpro® quality grade reagents for each step of nucleic acid-mediated viral vector-based manufacturing. 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.

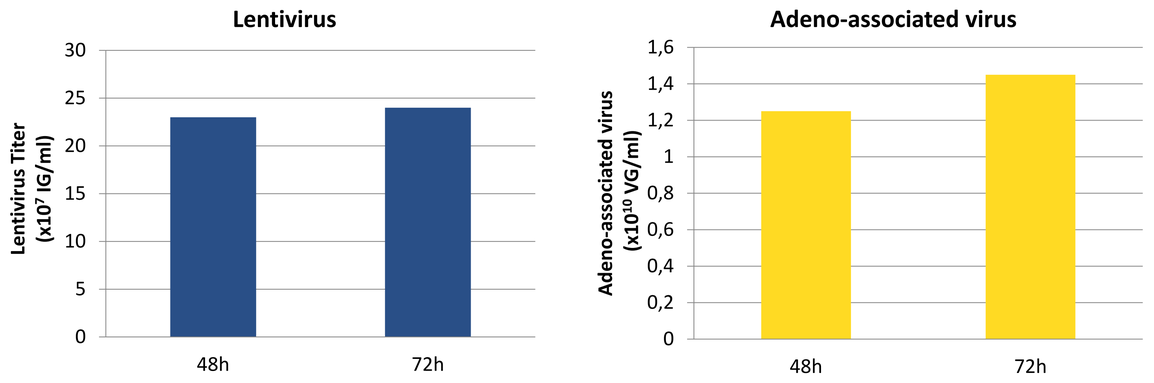


Reproducible virus titers with different grades of PEIpro®. Suspension HEK-293T cells were seeded at 1 x 10^6 cells/mL in FreeStyle™ F17 medium and transfected with PEIpro®, PEIpro®-HQ or PEIpro®-GMP reagents following the same protocol. AAV were produced with Helper Free Packaging System (Cell Biolabs) and titers were measured 72h after transfection using a GFP reporter gene expression.

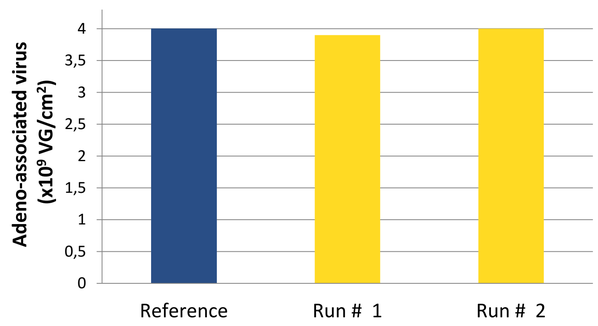
Gold standard for high virus production yields

Cell culture system	Vector	Cells	Titer
CS10® / CF10®	AAV	Adherent HEK-293, HEK-293T	10^{11}-10^{13} VG / ml
Fixed-bed bioreactor (iCELLIS®)	AAV	Adherent HEK-293T	10^{14}-10^{16} Total VG
Shaker Flask	AAV	Suspension HEK-293, HEK-293T	10^{9}-10^{10} VP / ml
Bioreactor	AAV	Suspension HEK-293, HEK-293T	0.8-1.5 x10^{9}-10^{10} VG / ml
10 cm dish/75 cm2	Lentivirus	Adherent HEK-293, HEK-293T	1-2 x10^{8} TU / ml
HYPERflask®/HYPERstack®	Lentivirus	Adherent HEK-293, HEK-293T	1-2 x10^{8} TU / ml
Shaker Flask	Lentivirus	Suspension HEK-293F, HEK-293T	2x 10^{7}-10^{10} VP / ml
Bioreactor	Lentivirus	Suspension HEK-293, HEK-293T	10^{7} IG / ml

PEIpro® is the reagent of choice for virus production runs in most cell culture systems in both adherent and suspension cells Irrespective of the cell culture-based system and production scale, PEIpro® and PEIpro®-HQ have led to efficient viral vector yields superior to 10^7 IG/mL and 10^9 VG/mL, respectively for lentiviruses and AAV.

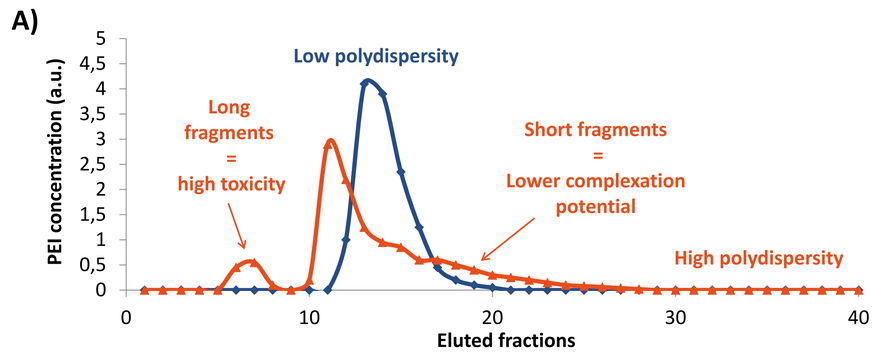


Lentivirus and AAV production in HEK-293T and HEK-293 cells grown in suspension in BalanCD® HEK293 (Irvine Scientific®). HEK-293T (lentivirus) and HEK-293 (AAV) cells were thawed directly into each medium and passaged every 3 to 4 days before going into a 2L benchtop bioreactor. Cells were seeded and cultured for 3 days before being transfected by PEIpro® (Polyplus). 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 Génethon).



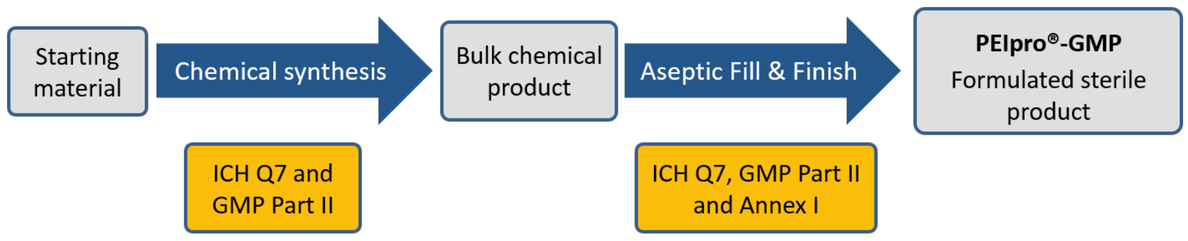
PEIpro® to simplify scale-up and to ensure reproducible virus production yields in iCELLis® Nano bioreactor. AAV-8 production in iCELLis Nano 0.8 m² (Reference) and 4 m². Triple PEIpro®-mediated transfection in F17 medium using 1.0 µg DNA/million cells and medium exchange with DMEM applied 5h post-transfection. Data are based on in situ cell lysis and AAV recovery at 72h post-transfection. qPCR analysis was performed on cell lysate. (Data kindly provided by Pall).

Optimized transient transfection for virus production



A) Optimization process of PEI polymer chemistry. Whereas long polymer fragments lead to cell toxicity and short fragments lead to lower complexation potential (in red), optimized PEI size with a low polydispersity index decreases toxicity, while increasing complexation potential (in blue) and reproducibility in transfection. **B) PEIpro® gives higher virus yields in comparison to CaPO4-based chemical transfection.** 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 flow cytometry of supernatants 48 h after transfection. **C) PEIpro® requires less reagent and similar to lower DNA amount compared to other PEIs.** Suspension HEK-293 cells were seeded at 1 x 106 cells/ml in serum free medium and transfected with PEIpro®, PEI "Max" and L-PEI 25 kDa (Polysciences, Warrington, PA) resuspended at 1 mg/ml. Luciferase expression was assayed 48 h after transfection using a conventional luciferase assay.

PEIpro®-GMP: highest quality grade PEI supplied in bags



Manufacturing process of PEIpro®-GMP. 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.

Conclusion: advantages of PEIpro® product range

- Best-in-class PEI-based transfection reagent for viral vector production
- Highest virus yields in producer cell lines (HEK-293 and derivatives, VERO, others)
- 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

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