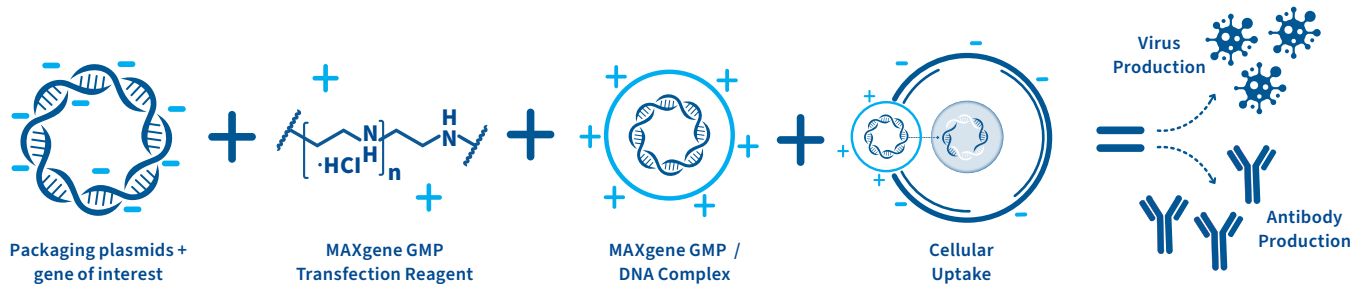


Transfection Reagents For Gene And Cell Therapy



Gene therapy is the transfer of genetically modified materials into a patient's tissues or cells with the goal of correcting the abnormal gene contributing to a particular disease. In recent years, biotherapeutics have emerged as effective treatments for a broad spectrum of diseases as numerous genes connected to disease and cellular processes have been identified as potential targets for therapeutic approaches (Sun 2017). It is estimated that every year hundreds of new biotherapeutic candidates are developed. Currently, one of the most critical limitations in developing effective gene therapies is the issue of delivery. These therapies require an efficient delivery system that is able to overcome extracellular barriers such as avoiding particle clearance mechanisms, targeting specific cells or tissues, and protecting the nucleic acid payload from degradation. These methods must also be able to navigate around the cellular barriers, including cellular uptake, endosomal escape, nuclear entry, and nucleic acid release (Durymanov 2018). Gene therapy vectors (viral vectors), used to achieve gene modification, are a viable "key" for an efficient and safe delivery strategy.

Although there are numerous viral vectors utilized in gene therapy development, Adeno-Associated Virus (AAV) and Lentivirus are used most commonly as they are highly effective and trusted modalities and they provide a means for efficient delivery of nucleic acid into cells *ex vivo*, *in vitro* and *in vivo* (Naso 2017). AAVs are particularly useful as gene therapy vectors because they can penetrate cells and introduce nucleic acids in such a way that the genetic material may be maintained in a stable configuration in target cells. Lentiviral vectors provide another option with their capacity for larger-sized nucleic acids and their ability to integrate with the host genome for long-term expression. These viruses are also able to introduce nucleic acids into specific host sites, providing a highly targeted approach. Because AAVs are not associated with pathogenic disease in humans, these vectors are able to deliver therapeutic proteins and agents to human patients with a low risk of adverse effects (Daya 2008). The newer generations of lentiviral vectors have also demonstrated minimal safety concerns in an increasing number of clinical applications (Milone 2018). As production of viral vectors is highly dependent on transfection, and with so many different options on the market today, it can be difficult to assess and find the optimum transfection reagent and delivery vehicle to attain high efficiency cell lines with low cytotoxicity for reliable clinical and research use.

Polyethylenimine (PEI) has an established history as a gold standard transfection reagent for both *in vivo* and *in vitro* gene transfer (Zakeri 2018). PEI is a polycationic polymer that works by forming a positively charged complex with nucleic acids that readily interacts with cell surfaces leading to endocytosis and transport into the cytoplasm, ultimately leading to delivery of the PEI:nucleic acid complex to the nucleus. PEI-mediated transfection has advantages over other available methods. PEI is well tolerated in many different cell types, including in cell lines commonly used for bioprocessing and has low cytotoxicity and high biocompatibility in both adherent and suspension cell culture systems (Sawant 2012).

Over the last decade, Polysciences has developed and introduced a wide range of unique, PEI-based transfection reagents in various quality grades (including cGMP & R&D grades) to be used as a critical raw material from process development through commercial biotherapeutics manufacturing. Founded in 1961, Polysciences has a long history of manufacturing unique, high-quality, specialty chemicals, and is a trusted partner of numerous reputed pharma and biotech customers developing gene/cell therapy products.

POLYSCIENCES PEI

With the exponential growth witnessed by the cell and gene therapy industry in recent years, the ability to manufacture high quality viral vectors to be used in clinical trials and commercial therapeutics at a large scale is critical. The overall success of the bioprocess system producing these products depends on various elements, with transfection reagents being one of the most significant factors. For companies who are invested in developing gene/cell therapies, it is important to choose the right partners with a proven track record of producing reagents for the successful launch of effective and reliable therapeutics. With 60 years of experience in manufacturing high-quality specialty chemicals and reagents for pharma, medical device, and biotech industries, Polysciences has proven itself to be a well-established, trusted partner that delivers high-quality, cost-efficient transfection reagents at any scale to help partners bring new therapies to market more rapidly, and ultimately, to a greater number of patients.

Transfection Reagents For Gene And Cell Therapy

Polysciences PEI has a high density of protonatable amino groups, with every third atom being amino nitrogen, which imparts a high buffering ability at nearly any pH. Hence, once inside the endosome, PEI disrupts the vacuole, releasing the genetic material into the cytoplasm. This ability to escape the endosome, as well as the ability to form stable complexes with nucleic acids, makes PEI a high efficiency gene delivery vector with decreased cytotoxicity. Polysciences offers a line of research grade PEI products (PEI MAX™ and Transporter 5™), as well as cGMP grade PEI products (MAXgene™ GMP), delivering cost-effective, trusted, and high-quality raw materials suitable for viral vector manufacturing at any scale.

Polysciences' produces PEI products with a number of advantages, making them a trusted choice by therapeutics manufacturers for their process development, through clinical trials and commercial manufacturing:

1. High-quality R&D and GMP grade material options
2. High transfection efficiency with low cytotoxicity
3. High reproducibility of viral/protein titers at any scale (small culture dishes to large scale bioreactors)
4. Flexible and suitable for stable and transient transfection in a wide range of cell lines, plasmids, and culture media formulations
5. All materials sourced and manufactured in the U.S. in an ISO-certified facility under an ISO 13485 Quality System
6. Cost-effective

PROCESS DEVELOPMENT TO COMMERCIALIZATION

Bioprocess Manufacturing Scale-up Sequence



NEW, MAXgene GMP powder (cat# 26435)

Polysciences offers a range of high-quality transfection reagents aiding with a smooth transition from process development through clinical trials and commercial manufacturing of viral vectors and proteins. Polysciences' R&D grade PEI MAX has been used by academic and industry customers for over a decade and has become widely cited throughout the literature. Polysciences has also launched Transporter 5, a more convenient version of the trusted PEI MAX in a ready-to-use, stable liquid formulation.

Developing a clinical-grade product according to FDA guidelines is a complex process with sourcing cGMP-grade raw materials being one of the most critical pieces to ensure the safety of the product for use in patients. Polysciences launched its first GMP transfection reagent, MAXgene GMP, in 2019 following strict cGMP practices, including stringent formulation, manufacturing, and quality control (QC) processes to ensure that the product always meets established specifications of identity, potency, purity, traceability, and safety with exceptional lot-to-lot reproducibility. With this latest addition, Polysciences could now offer a suite of transfection reagents to meet industry needs for all phases of biotherapeutics development and manufacturing.

HIGH REPRODUCIBILITY AND TRANSFECTION EFFICIENCY

PEI MAX and Transporter 5 are designed to be used for viral vector production process development. For clinical trial and commercialization, Polysciences recommends MAXgene GMP, which is available in both powder and liquid formats that meet the quality and regulatory requirements of gene/cell therapy raw materials. All three products are fully hydrolyzed, easy to handle, and deliver higher transfection efficiency. Polysciences' linear PEI reagents are widely cited, with publications demonstrating their suitability for process development with the goal of scaling viral vector manufacturing in a cost-effective way.

Polysciences PEI products have high transfection efficiency from lot to lot, as shown in Figure 1. This high transfection efficiency translates to high titer yields in both small- and large-scale cultures. Figure 2 from Griger et al demonstrates high titer yields of rAA8 CM-eGFP from HEK293 cells in large scale bioreactors.

Transfection Reagents For Gene And Cell Therapy

The optimal PEI and DNA concentrations/ratios for high transfection efficiency can differ depending on the cell type and other assay parameters. A comparative analysis done by Gu *et al* (2018) between Polyplus® PEIpro® and Polysciences PEI MAX on triple transfection for AAV production in suspension HEK293 cells showed a ~3-fold titer increase using PEI MAX compared to PEIpro.

The assay conditions used by Gu *et al* (2018) are shown below and to the right:

PEI MAX OPTIMAL CONDITIONS: TRIPLE TRANSFECTION FOR AAV PRODUCTION IN SUSPENSION HEK293 CELLS	
pHelper: pRC: pGOI (molar ratio)	01:01:02
DNA concentration (mg/ml)	1
Cocktail volume	10%
Reagent: DNA	02:01
Cell density (vc/ml)	1.00E+06

Polysciences' PEI Products Deliver High Transfection Efficiency Consistently Across Different Lots

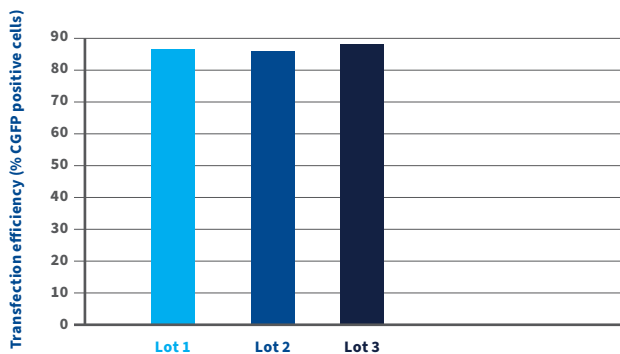


Figure 1: Suspension HEK-293T cells were seeded at 1 x 10⁶ cells/mL and transfected with three different lots of MAXgene GMP/Transporter 5 with a GFP-expressing plasmid. Transfection efficiency was measured 48 hours post-transfection by flow cytometry.

Polysciences' PEI Max Product Yields High Titers Of AAV After 72 Hours Culture In A Large Bioreactors

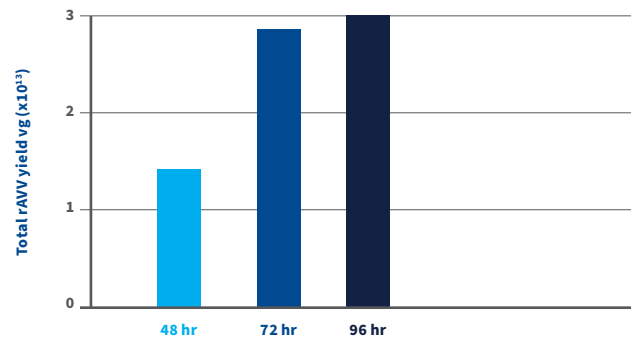


Figure 2: Flasks containing 30mL cultures of suspension HEK293 cells at 1x10⁶ cells/mL were transfected to produce rAAV8 CMV-eGFP. rAAV8 was harvested from the media 48 hours post-transfection with increasing amounts of vector found in the media over time (Griger 2015).

Low Toxicity Demonstrated In Cells Across Three Lots Of Polysciences' MAXgene GMP Product

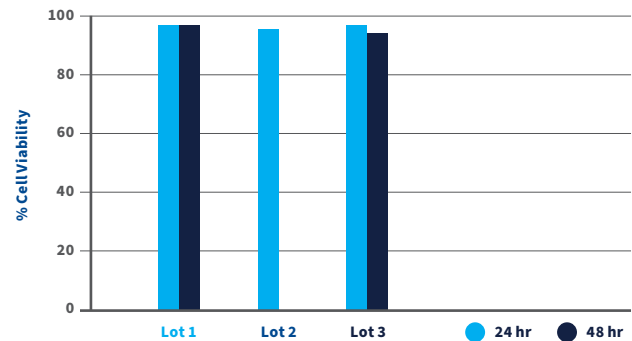


Figure 3: Suspension HEK-293T cells were seeded at 1 x 10⁶ cells/mL and transfected with three different lots of MAXgene GMP with adalimumab-expressing plasmid. Cell viability was checked by a ViCell cell counter 24 and 48 hrs. s post-transfection. Data show high viability for all transfection reactions, indicating low or no toxicity for the reagent lots teste.

Transfection Reagents For Gene And Cell Therapy

LOW CYTOTOXICITY

It is critical to optimize all protocol parameters to yield highly efficient bioprocesses. Appropriate selection of a transfection reagent is especially important, as it has a significant impact on the overall process and final product yield. It is also critical to maintain high cell viability and low toxicity throughout the process.

There are several studies that observed high transfection efficiencies combined with low cytotoxicity when using Polysciences' linear PEIs (Aravindan 2009, Dai 2011, Nimesh 2007). PEI MAX is nearly fully deacylated, which contributes to the increased efficiency while making it a less cytotoxic transfection reagent.

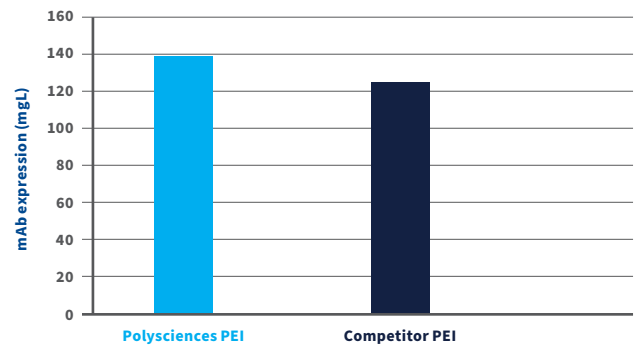
Delafosse et al (2016) investigated the cytotoxicity of three PEI-based transfection reagents (PEI MAX, PEI 25k, and PEIpro) in 293-6E and CHO-3E7 cell lines using the PI fluorescent dye exclusion assay and observed PEI MAX as the least toxic reagent for both cell lines compared to PEIpro. Similar results are seen with Transporter 5 and MAXgene GMP. Figure 3 shows high cell viability in HEK 293T cells after transfection of three different lots of MAXgene GMP.

SCALABILITY & FLEXIBILITY

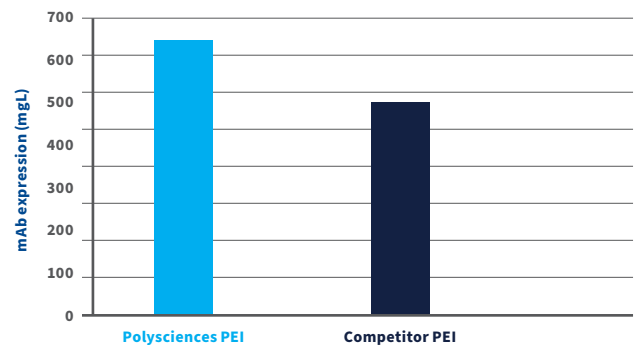
Polysciences' PEI reagents are suitable for a wide range of cell line and cell types. They are suitable for stable and transient transfection, ensuring high viral/protein titers in both adherent and suspension cultures.

As many viral vector manufacturing processes use suspension cell lines derived from Human Embryonic Kidney (HEK 293) and Chinese Hamster Ovary (CHO) cells, the figure to the right shows data comparing performance of Polysciences' PEI compared to a competitor PEI product in both cell lines.

Performance in CHO Cells



Performance in HEK Cells



*Expression of mAb B72-3, 8 days post-transfection using Gibco Freestyle F17 cell culture media. For more information, see the following publication: Delafosse, L., Xu, P. & Durocher, Y. *Comparative study of polyethylenimines for transient gene expression in mammalian HEK293 and CHO cells*. Journal of Biotechnology 227,103–111 (2016). doi:10.1016/j.jbiotec.2016.04.028

CONCLUSION

Biotherapeutics have emerged as effective treatments for a range of diseases, and when examining the final biotherapeutic, it is important to reflect back on the entirety of the development process. The continuum of development, with quality and consistency along the way, results in a superior final biotherapeutic that is safe and effective in patients. As an important manufacturing partner for cell/gene therapy manufacturers for more than a decade, Polysciences understands this continuum and the need for quality raw materials to navigate this continual development process from the early stages through pivotal trials and final drug delivery to patients.

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ORDERING INFORMATION

Please contact us at info@polysciences.com for our products produced in accordance with 21 CFR 210, 211

CAT #	DESCRIPTION	UNIT SIZE
26406-1	MAXgene® GMP Transfection Reagent (solution)	1 L
26435-1	MAXgene® GMP Transfection Reagent (powder)	1 g
26008-1A	Transporter 5® Transfection Reagent	1 ml
26008-5	Transporter 5® Transfection Reagent	5 ml
26008-50	Transporter 5® Transfection Reagent	50 ml
24765-1	PEI MAX® - Transfection Grade Linear PEI	1 g
24765-100	PEI MAX® - Transfection Grade Linear PEI	100 mg