

March 1, 2022

**Keywords or phrases:**

HEK293 cells, HEK media, gene therapy, serum-free culture, suspension culture, viral vector

# The Journey to AAV Production in Suspension – Scaling-Up Your Process

**Sandra Klausing**

Sartorius Xell GmbH, Waldweg 21, 33758 Schloss Holte-Stukenbrock

Correspondence

Email: [sandra.klausing@sartorius.com](mailto:sandra.klausing@sartorius.com)

## Abstract

The efficient production of adeno-associated viral vectors (AAVs) starts with scalable upstream manufacturing processes. Here, we present a simple protocol for scaling up AAV production using HEK293 host cells cultured in suspension and under serum-free conditions. We tested cell viability and the genomic titer of AAV-8 and AAV-2 serotypes.

# Introduction

Adeno-associated viral vectors (AAVs) are among the most widely used vectors for gene therapy applications (1). A robust AAV manufacturing process relies on productive host cells, and typically HEK293 cells are the cell line of choice.

HEK293 cells are traditionally grown in adherent, monolayer cultures. Transitioning cells to suspension culture is the first step in the journey towards a scalable AAV production process.

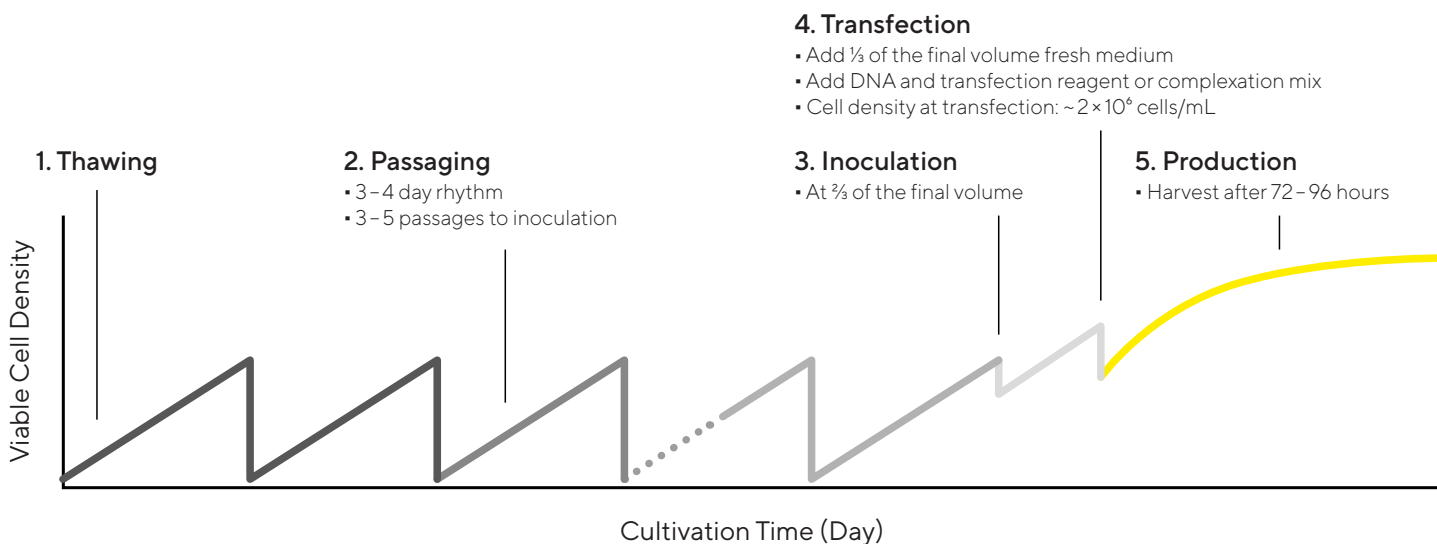
In this application note, we demonstrate the performance of our easy protocol for the production of two AAV serotypes. We applied this procedure to an initial scale-up step from shake flasks to 2 L benchtop bioreactors, and measured both cell viability and AAV titer.

# Materials

HEK293 cells were cultured in HEK ViP NX Medium. No growth factors were added, but some HEK293 cell lines require supplementation and adding, for example, insulin or IGF, can increase AAV titers.

Our transfection mix was PEI MAX<sup>®</sup> (Polysciences) with a 2-plasmid system for AAV-2 and AAV-8 (PlasmidFactory) using low total DNA amounts of 1 µg/mL culture at a 4:1 ratio in fresh medium.

**Figure 1:** Example Procedure for Seed-Train and Production Process.



# Methods

HEK 293 cells were thawed and expanded over at least three passages. Small-scale processes were performed in 30 mL volume in 125 mL Erlenmeyer shake flasks. The initial scale-up step was performed in 2 L “stirred-tank” benchtop bioreactors, representing a robust scale-down model for larger controlled systems.

We inoculated the production process at a density of 1.5–2 million cells/mL at half (or more) of the final cultivation volume. The cells were allowed to equilibrate in the system for one day, and then fresh medium at half (or less) of the final volume and the pre-complexed transfection mix were added. The addition of medium and transfection mix should set viable cell density to 1.5–2.5 million cells/mL.

Samples were taken daily from the production processes to analyze viable cell density, viability, transfection efficiency, and AAV titers. Final harvest was done at 96 h (AAV-2) and 120 h (AAV-8) post transfection.

Genomic AAV-8 titers were determined via qPCR from culture supernatants without cell lysis, but after DNase I and Proteinase K digest.

Genomic AAV-2 titers were determined via qPCR from culture supernatants and in lysed cell from cell pellets but after DNase I and Proteinase K digest.

# Results

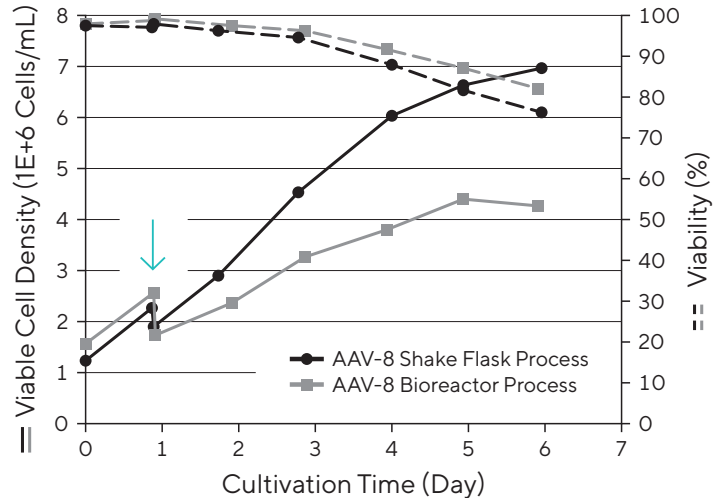
We applied our protocol to the production of two AAV serotypes: AAV-8 and AAV-2.

## Case Study 1: Scale-Up Step for AAV-8

HEK293 cultures for AAV-8 production were inoculated from a common pre-culture at around 1.5 million cells/mL and diluted with fresh media to around 2 million cells/mL for transfection on day 1 (Figure 2, green arrow).

While the maximum cell density in the shake flask reached 7 million cells/mL on day 5 (120 h), the bioreactor culture showed a lower maximum cell density of 4.4 million cells/mL (Figure 2). Cell viability was comparable between the two serotypes (Figure 2).

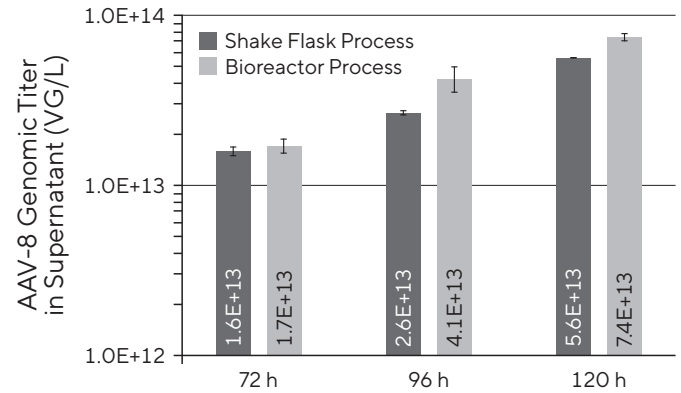
**Figure 2:** Cell Density and Viability in AAV-8 Production



Note. Cell density and viability in shake flask and bioreactor processes from inoculation (day 0) to transfection (day 1) and harvest (day 4). The green arrow indicates the time of transfection.

Genomic AAV-8 titers were consistently higher than AAV-2 titers between shake flask and bioreactor cultures (Figure 3).

**Figure 3:** Genomic Titers in AAV-8 production

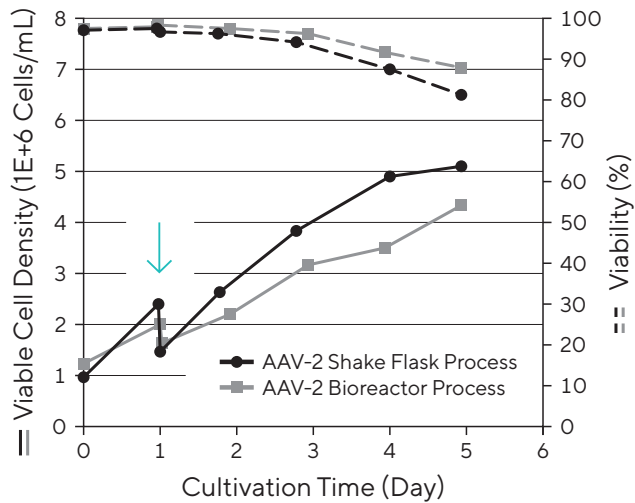


Note. AAV-8 genomic titers in culture supernatants.

## Case Study 2: Scale-Up Step for AAV-2

HEK293 cells for AAV-2 production were inoculated from a common pre-culture at around 1-1.5 million cells/mL and diluted with fresh media to around 1.5 million cells/mL for transfection on day 1 (Figure 4, green arrow).

**Figure 4:** Cell Density and Viability in AAV-2 Production



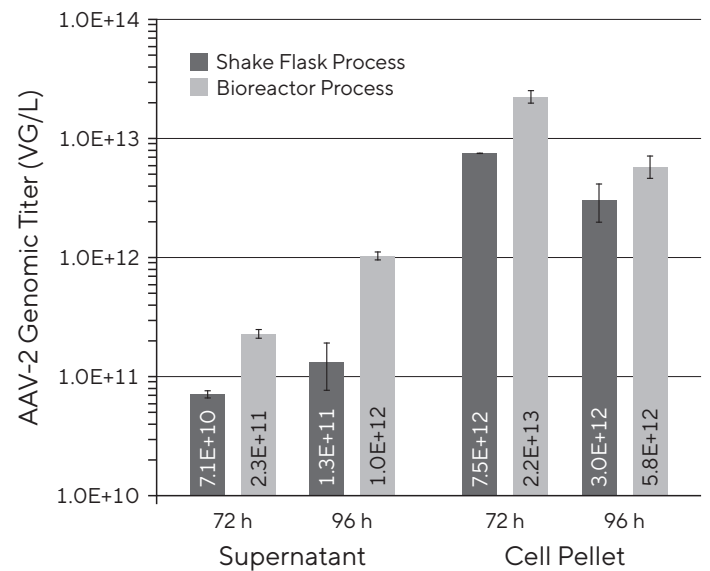
Note. Cell density and viability in shake flask and bioreactor processes from inoculation (day 0) to transfection (day 1) and harvest (day 5). The green arrow indicates the time of transfection.

Similar to AAV-8 processes, the bioreactor process also showed lower cell growth during AAV production, with higher viability at harvest (Figure 4).

Unlike some AAV serotypes such as AAV-8, AAV-2 is highly retained within the cells (2). Therefore, genomic titers were measured in lysed cells as well as supernatants. AAV-2 titers from lysed cells decreased with prolonged cultivation.

In contrast, titers in the supernatant increased over time. Similar to our AAV-8 results, AAV-2 titers were higher in the bioreactor process compared to the shake flask process for all samples.

**Figure 5:** Genomic Titers in AAV-2 Production



Note. AAV-2 genomic titers in culture supernatants and cell pellets (lysed cells).

# Discussion

Here, we presented a straightforward approach to upstream AAV production and scale-up. There are various parameters that could require optimization during the scale-up of the upstream AAV production process.

- The seed-train and optimal passage number at transfection should be optimized to fit the final process steps. The passage number should be >3, but <10 after thawing, unless otherwise tested.
- The inoculation cell density should be fine-tuned to deliver high cell densities without running into limitations.
- The time of transfection should be carefully determined to ensure the cells are both equilibrated in the controlled environment and in an exponential growth phase.
- Different washing and feeding regimes should be tested. The ratio of fresh medium introduced before transfection can influence transfection efficiency and viral titers.
- pH profiles, temperature shifts, and other environmental perturbations should be carefully managed when switching between uncontrolled and controlled systems.

# Conclusion

## A Straight-Forward Approach Allows Easy Scale-Up

In this application note, we demonstrate a simple procedure for scaling up AAV-8 and AAV-2 production processes. We successfully scaled up an uncontrolled shake flask culture to a bioreactor system, achieving higher titers in the controlled bioreactor process in HEK293 cells.

HEK ViP NX Medium was used in both processes. This medium is chemically-defined, serum-free, and animal component-free. It's composition was the best fit for our HEK 293 cell line used and enabled rapid set up of the scale-up experiments. These properties give consistent results and maximum control over the AAV production process, instrumental to the success of transfer across scales and vessels in our experiments.

# References

- [1] Merten, O.-W., Schweizer, M., Chahal, P., & Kamen, A. A. (2014). Manufacturing of viral vectors for gene therapy: part I. Upstream processing. *Pharmaceutical Bioprocessing*, 2(2), 183–203. <https://doi.org/10.4155/pbp.14.16>
- [2] Vandenberghe, L. H., Xiao, R., Lock, M., Lin, J., Korn, M., and Wilson, J. M. (2010). Efficient serotype-dependent release of functional vector into the culture medium during adeno-associated virus manufacturing. *Human Gene Therapy*, 21(10), 1251–1257. <https://doi.org/10.1089/hum.2010.107>

## Germany

Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen  
Phone +49 551 308 0

Sartorius Xell GmbH  
Waldweg 21  
33758 Schloss Holte-Stukenbrock  
Phone +49 521 96989 200

## USA

Sartorius Stedim North America Inc.  
565 Johnson Avenue  
Bohemia, NY 11716  
Toll-Free +1 800 368 7178

 **For further information, visit**  
[www.sartorius.com](http://www.sartorius.com)