A robust system for high-titer, platform-agnostic AAV production in HEK293 cell lines

Lonza
Specialized Modalities

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Abstract

To maximize the results of viral titer and full capsids, it is necessary for any gene therapy program to undertake systematic process development and optimization of AAV production. Development and optimization strategies have been shown to increase titer and enrich full capsids through screening both components of the production process as well as fine-tuning the stoichiometries and delivery methods of these components. Improving these critical metrics is a multi-parameter problem, spanning all process components.

Choice of cell culture medium is foundational to productivity and efficiency of packaging since this media is the environment in which all upstream steps of AAV production take place. A chemically-defined media that is free from animal origin components is table stakes for further manufacturing (GMP) programs as it supports consistency and regulatory compliance. However there are unique performance characteristics to media that provide research and commercial advantages for the critical quality metrics of titer and full capsids mentioned above:

1. Compatibility with mostly fixed process components such as viral serotype and plasmid/gene of interest
2. Flexibility with variable process components such as cell line, transfection reagents, and enhancers or additives that increase production (Figure 1)
3. Baseline (non-optimized) performance that meets or exceeds industry standards

By fulfilling these three key characteristics, it is possible for a cell culture media system to enable multi-fold titer and full capsid gains in AAV production.

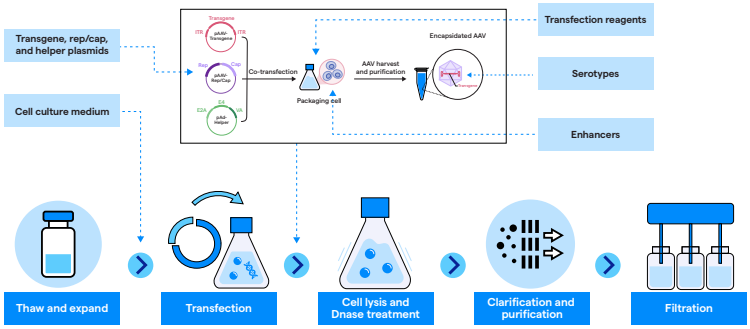


Figure 1.
Schematic representation of AAV production components surveyed in this study.

Materials and methods

Cell culture

Human Embryonic Kidney (HEK) 293 cells were thawed directly into TheraPEAK 293-GT® Medium and cultured in 125 mL shake flasks for at least 3 passages after thaw.

Crude lysate harvest

72 ± 2 hours after transfection, cell culture was collected and lysed with lysis buffer stock prepared in house. Lysate was centrifuged at 1000 RCF for 10 minutes

Cell culture

Transfections were performed with 30 mL of diluted cell culture from above for each flask. For flasks with TheraPEAK 293-GT® Medium, TheraPEAK 293-GT® AAV Supplement was added to cell culture within one hour prior to transfection. Research-grade helper plasmids, transfer plasmids and cis plasmids (GFP) were either Lonza Xcite® AAV Transient Transfection Plasmids.

Commercially available transfection reagents and AAV enhancers were acquired from respective vendors.

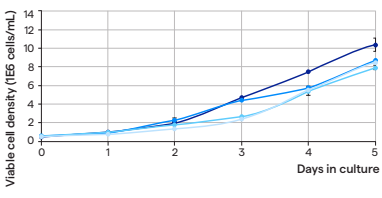
Crude lysate harvest

AAV titer was assessed with droplet-based digital PCR using industry standard protocols through direct quantification of copy numbers of gene of interest (GFP).

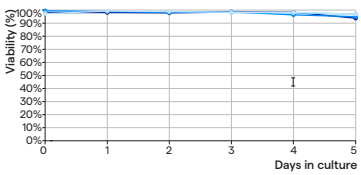
Total AAV capsid titer in crude lysate was quantified with an automated immunoassay system following vendor's recommended protocol. Full/empty capsid ratio was estimated by dividing AAV genome titer (quantified with droplet-based digital PCR) by total capsid titer.

Results

Cell growth



Cell viability

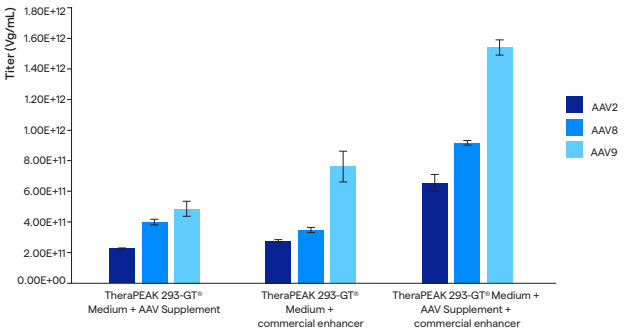


Across four different HEK293 cell lines, Lonza's TheraPEAK 293-GT® Media System supports robust cell growth. Some cell lines experienced up to 59% higher density after 5 days in TheraPEAK 293-GT® Media System than in their cloning/adaptation media (A).

TheraPEAK 293-GT® Media System maintained a high viability of ≥95% across the same four different HEK293 cell lines, demonstrating a superior ability to support proliferation needed for AAV production (B).

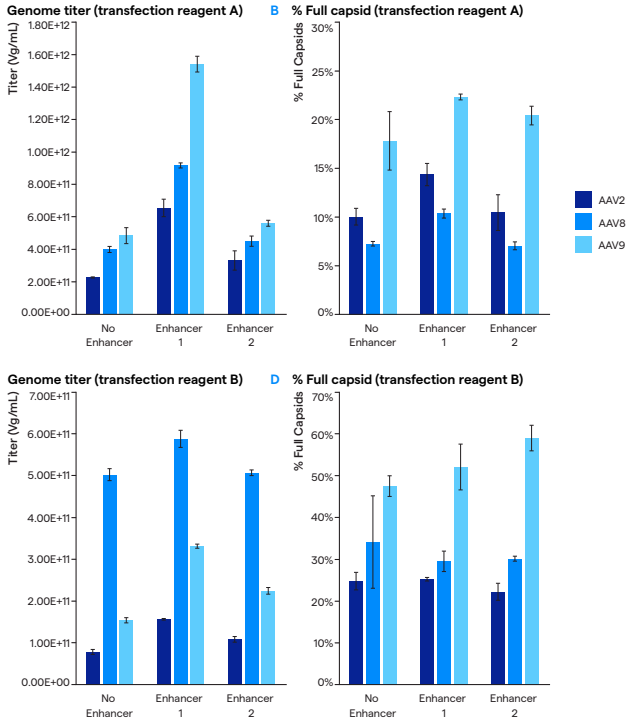
Results

Synergistc effect of AAV supplement



Addition of TheraPEAK 293-GT® AAV Supplement during transfection supports increased AAV titer as compared to using a commercial enhancer alone. Here, use of the complete TheraPEAK 293-GT® Media System delivers a ~3x increase in AAV titer whereas a commercial enhancer alone delivers only a ~2x increase above baseline performance.

High performance across process development components



TheraPEAK 293-GT® Media System helps deliver favorable AAV titer (A, C) and favorable full capsid ratios (B, D) across multiple transfection reagents, enhancers, and viral serotypes, making it ideal for commercial AAV production. This supports the benefits of easy integration into existing workflows, and efficacy for process development for a variety of production platforms

Conclusion

TheraPEAK 293-GT® Media System enables success for AAV development programs through robust cell expansion, increased titer, and favorable full capsid ratios. This performance has been validated across a survey of viral serotypes, transfection plasmids, transfection reagents, and AAV enhancers, as well as benchmarked against a competitor AAV media and complete production platform. The aforementioned flexibility and performance demonstrate that Lonza's TheraPEAK 293-GT® Media System is ideal for commercial gene therapy process development and cGMP-compliant manufacture.

Learn more.

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