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Specialized Modalities

A flexible cell culture media for high titer production of AAV in HEK 293

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Through research and clinical successes, adeno-associated virus (AAV) has emerged as a leading modality for *in vivo* delivery of genetic information, particularly in the field of gene therapy. As with any therapeutic field, upstream productivity and efficiency of the drug development process influences many aspects of downstream clinical and commercial success. In the world of AAV, these metrics are volumetric productivity as measured by titer (typically measured by qPCR or droplet-based digital PCR) and packaging efficiency as measured by the distribution of complete genome-containing ("full") versus partially filled or empty capsids[1].

The adverse effects of poor performance in these metrics puts lead candidates at risk by inflating operational and capital expenditures or scuttling clinical trials. From an operations perspective, low titers increase both cost pressures from additional starting material and purification expenses and capacity constraints from the need to increase production scale or batches; from a therapeutic perspective, a low distribution of full capsids can dilute potency and increase immunogenicity[2]. In fact, U.S. FDA chemistry, manufacturing, and control (CMC) guidance highlights the need to define and control these attributes during therapeutic development[3]. Accordingly, there are commercial, scientific, and regulatory imperatives to increase these metrics in an AAV production process.

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[4]. 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
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.

Materials and methods

Cell culture

Human Embryonic Kidney (HEK) 293 cells were thawed directly into either TheraPEAK 293-GT® Medium or commercial media from competitor commercial organizations and cultured in 125 mL shake flasks for at least 3 passages after thaw. In TheraPEAK 293-GT® Medium, cells were seeded at 0.5×10^6 cells/mL and cultured with shaking at 37±2 °C (≥80% relative humidity, 5% CO_2) for 3 to 4 days for every passage. For commercial media, vendor recommendations were followed. Three days before transfection, cells were sub-cultured with seeding density of 0.7×10^6 cells/mL. On the day of transfection, cells were then diluted to 3×10^6 cells/mL with TheraPEAK 293-GT® Medium or other commercial media.

Transfection

Transfections were performed in 125 mL shake flasks 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 at a ratio of 1 mL per 30 mL culture. Transfection was performed with a commercially available reduced serum medium as the complexation buffer, following a complexation volume ratio of 10% v/v (3 mL added to 30 mL of culture). Research-grade helper plasmids, transfer plasmids and cis plasmids (GFP) were either Lonza Xcite® AAV Transient Transfection Plasmids or acquired from another leading commercial organization. 1:1:1 molar ratio was used for the transfection of the three plasmids. Commercially available transfection reagents and AAV enhancers were acquired from respective vendors. Vendor's recommendations on DNA amount per unit of cell, DNA to transfection ratio, enhancer addition volume and complexation time were followed.

Crude lysate harvest

72 ± 2 hours after transfection, cell culture was collected and lysed with lysis buffer stock prepared in house. Lysis buffer was added at a ratio of 133 µL per 1 mL of harvested culture and incubated at 37 °C for 2 hours with shaking. Lysate was centrifuged at 1000 RCF for 10 minutes.

AAV genome titer and full to empty ratio quantification

AAV titer was assessed with droplet-based digital PCR using industry standard protocols through direct quantification of copy numbers of gene of interest (GFP). Harvested crude lysate was processed by industry standard protocols to remove free-floating DNA and digest capsid proteins. Reaction mixtures were prepared, and droplet-based digital PCR was then performed following manufacturer's protocol.

Capsid full/empty ratio analysis

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

TheraPEAK 293-GT® Medium supports the expansion of various HEK 293 cell lines

Four different, commercially available HEK 293 cell lines were cultured in TheraPEAK 293-GT® Medium without any feed and supplementation for 3 passages and seeded at 0.5×10^6 cells/mL for a 5-day cell growth evaluation. All 4 cell lines were able to proliferate to beyond 7.5×10^6 cells/mL on day 5 and maintained > 95% viability throughout the 5-day period (Figure 1). In addition, we also observed that our medium can support the proliferation of these cell lines to cell density up to 12×10^6 cells/mL by day 7 (data not shown). This demonstrates the capability of TheraPEAK 293-GT® Medium to support robust cell expansion to levels well beyond the need for AAV production purposes.

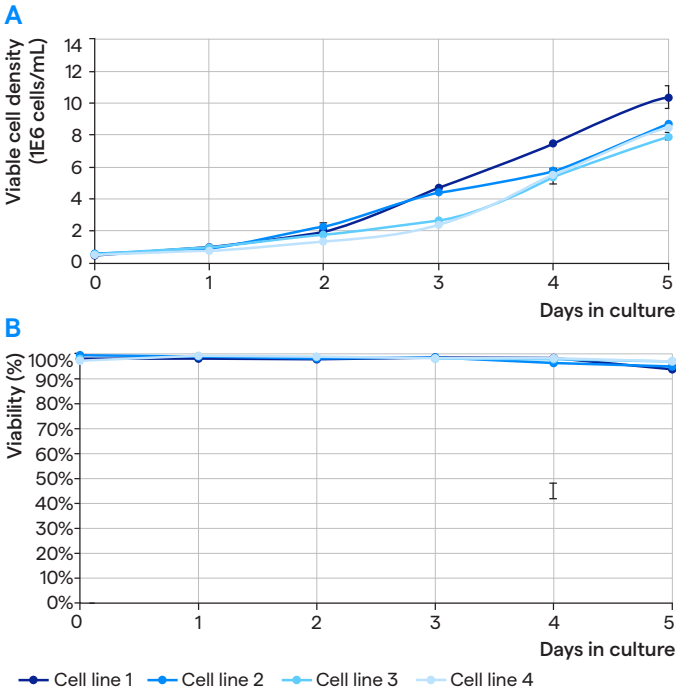

Figure 1.
Viable cell density (A) and viability (B) of four HEK 293 cell lines cultured in TheraPEAK 293-GT® Medium for 5 days.

TheraPEAK 293-GT® AAV Supplement improves AAV productivity and exhibits synergistic effect with commercial enhancer

Performance of TheraPEAK 293-GT® Medium and AAV Supplement and their interaction with commercial enhancer was evaluated by the yield of AAV2, 8 and 9 with Lonza Xcite® AAV Transient Transfection Plasmids, a commercial HEK 293 cell line, and commercial transfection reagent A. With TheraPEAK 293-GT® Medium, TheraPEAK 293-GT® AAV Supplement demonstrated performance comparable to that of commercial enhancer in AAV2 and AAV8 productivity with slightly lower titer of AAV9 (Figure 2). Interestingly, when both the AAV supplement and commercial enhancer are incorporated, about 100% increase in productivity was observed for all three serotypes, demonstrating the synergistic effect in boosting AAV productivity and the potential of TheraPEAK 293-GT® AAV Supplement to be used with other enhancers.

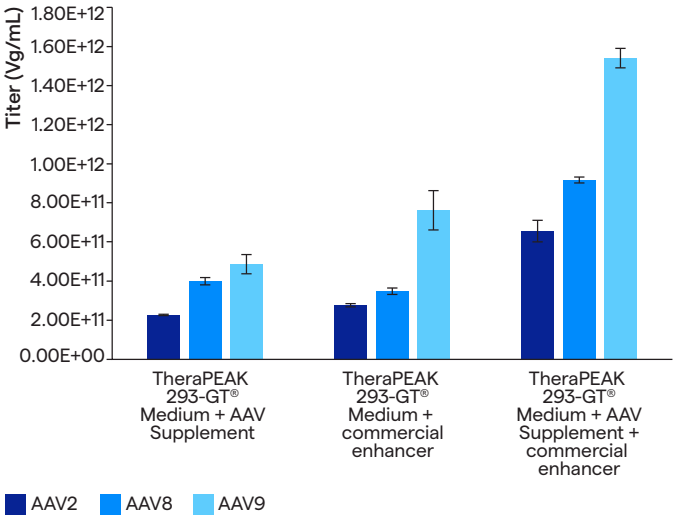

Figure 2.
Genome titer of harvested crude lysate produced with commercially available HEK 293 cell line in TheraPEAK 293-GT® Medium with and without TheraPEAK 293-GT® AAV Supplement and a commercial enhancer.

TheraPEAK 293-GT® Media System demonstrates flexibility of incorporating different commercial transfection reagents, enhancers and plasmids for achieving high AAV productivity

Flexibility of incorporating different transfection reagents and enhancers in process development is a key advantage of TheraPEAK 293-GT® Media System. To demonstrate such flexibility, two commercially available transfection reagents and AAV enhancers were used in AAV production with TheraPEAK 293-GT® Media System without process optimization for three common AAV serotypes 2, 8 and 9. When using transfection reagent A, titers of all three serotypes are above 2E+11 viral genome/mL in crude lysate and % full capsid above 5%, with enhancer 1 exhibiting particularly high yield with comparable % full capsid (Figure 2A and 2B). For transfection reagent B, the use of enhancer 1 and 2 demonstrated certain performance increase (Figure 2C). Although genome titers are in general lower than that with transfection reagent A, overall % full capsid values are much higher (Figure 2D), suggesting that transfection reagent has significant impact on packaging efficiency. These results show flexibility of TheraPEAK 293-GT® Media System to deliver high viral titer and favorable % full capsids across different transfection reagents, enhancers, and AAV serotypes, demonstrating high compatibility and customizability of TheraPEAK 293-GT® Media System.

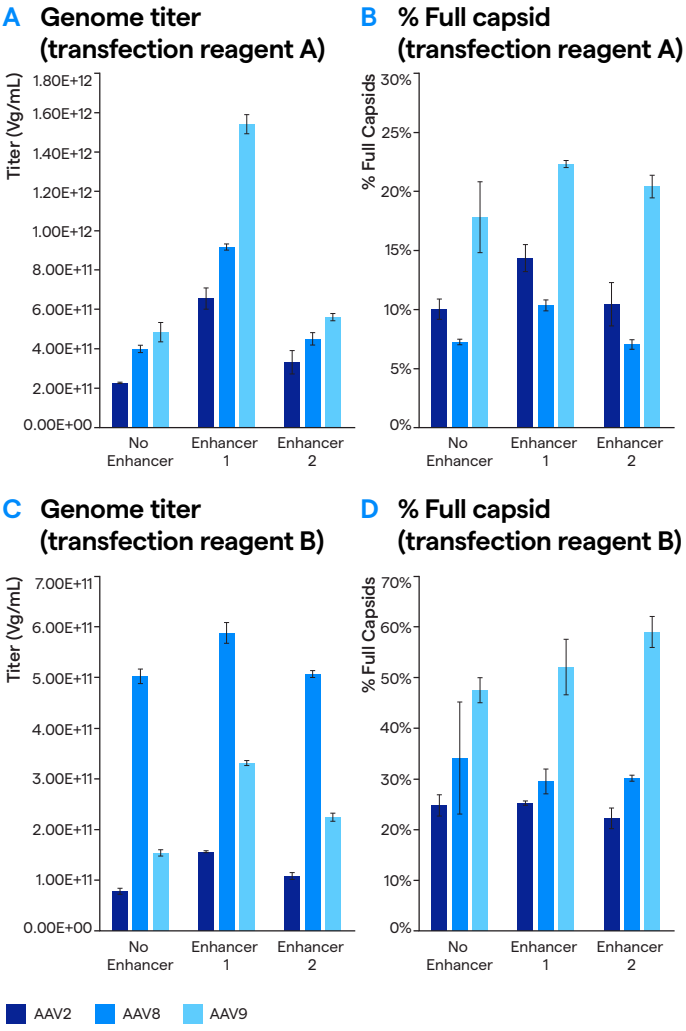

Figure 3.
Flexibility of TheraPEAK 293-GT® Media System demonstrated by AAV genome titer and % full capsid with the usage of commercial transfection reagent A (A and B) and transfection reagent B (C and D) with 2 different commercial and AAV enhancers. Lonza Xcite® AAV Transient Transfection Plasmids were used for transfection.

In addition, the use of a different set of plasmids did not impact the high performance demonstrated by the TheraPEAK 293-GT® Media System. With the same process parameters, AAV9 yield with a standard commercial plasmid set is slightly lower than that with Lonza Xcite® AAV Transient Transfection Plasmids (Figure 4). Overall, the high AAV yield with combinations of different transfection reagents, enhancers and plasmids without process optimization indicates that TheraPEAK 293-GT® Media System can be readily and easily implemented to process development of AAV production for a variety of production platforms.

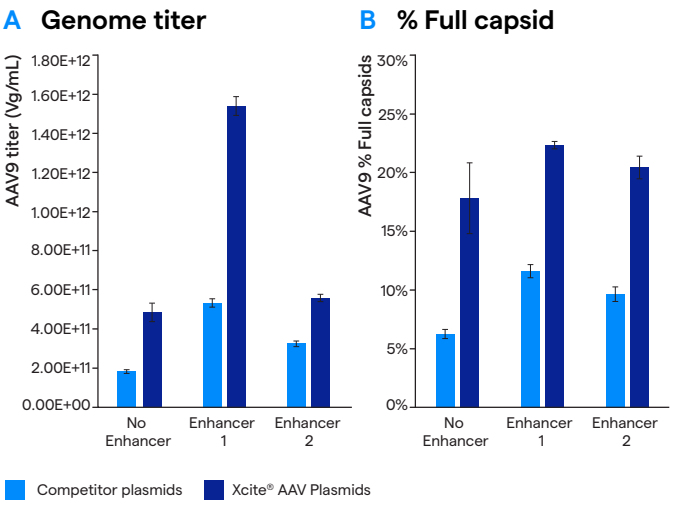

Figure 4.
Genome titer (A) and % full capsid (B) of AAV9 produced from Lonza Xcite® AAV Transient Transfection Plasmids or competitor plasmids with TheraPEAK 293-GT® Media System. Commercial HEK293 cell line and transfection reagent A was used with no AAV enhancer, enhancer 1 and enhancer 2.

TheraPEAK 293-GT® Media System exhibits high potential in developing AAV production platforms with superior performance and efficacy compared to other AAV production medium and system

A different AAV medium was used in cell culture and transfection with same process parameters applied, and the resulting AAV titers were compared against that with TheraPEAK 293-GT® Media System. Without the addition of commercial enhancer, TheraPEAK 293-GT® Media System yielded more than 1.8× higher titers for all three serotypes (Figure 5). The inclusion of enhancer is able to further increase AAV titer with an additional 3- to 7-fold improvement.

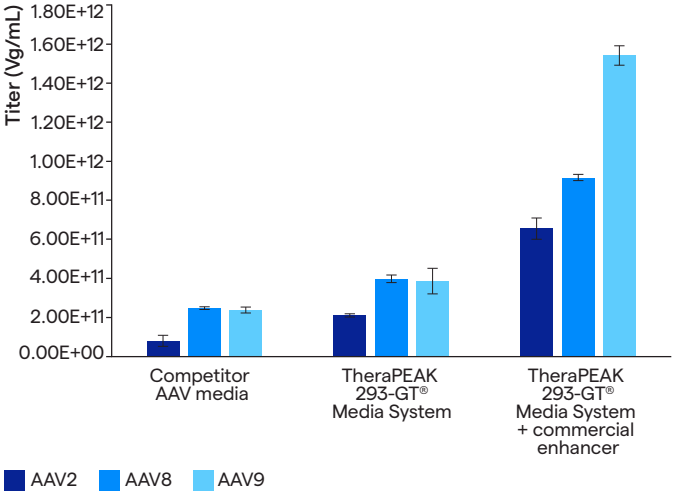

Figure 5.
Genome titer of AAV2, 8 and 9 produced with a competitor AAV media and TheraPEAK 293-GT® Media System with and without commercial enhancer. Lonza Xcite® AAV Transient Transfection Plasmids were used in both a competitor AAV media and TheraPEAK 293-GT® Media System. Transfection reagent A was used in both cases.

TheraPEAK 293-GT® Media System was also compared against a competitor AAV production platform. When implemented together with commercial transfection reagents and enhancer[1], the resulting AAV genome titer from TheraPEAK 293-GT® Media System greatly exceeds that from the competitor AAV platform (Figure 6A), highlighting the optimal performance of TheraPEAK 293-GT® Media System in supporting manufacturing of AAV products.

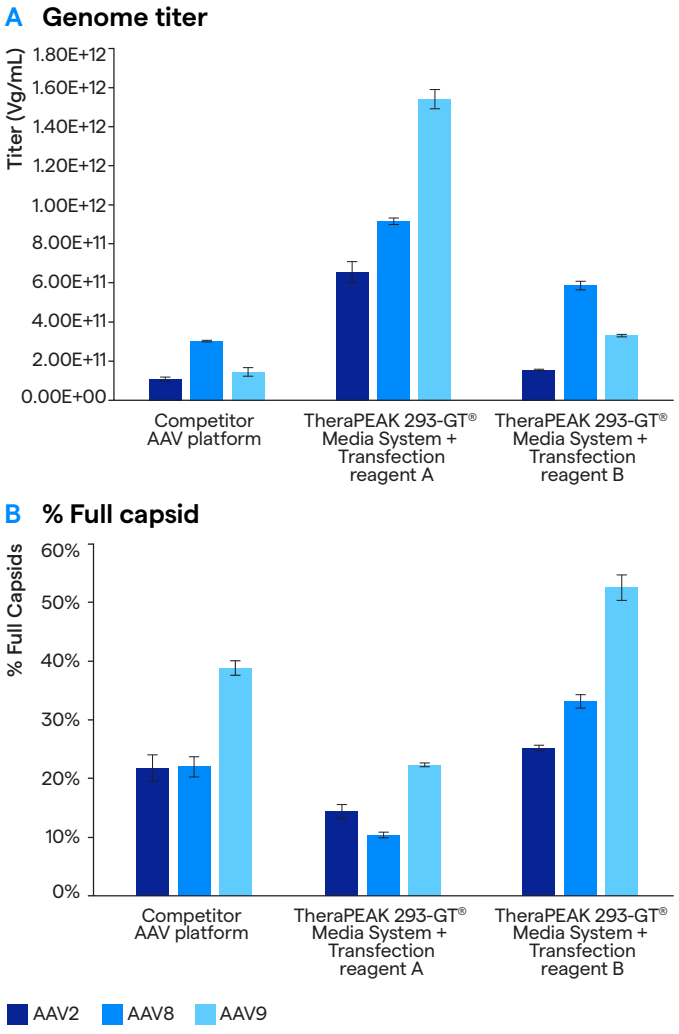

Figure 6.
Performance comparison for AAV 2, 8, and 9 genome titer (A) and % full capsid (B) between a competitor AAV platform and TheraPEAK 293-GT® Media System with commercial transfection reagents A and B and commercial enhancer 1.

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.

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