# The Viral Vector HEK Media Panel addresses HEK293 cell lineage diversity in AAV production through basal screening

## Introduction

Gene therapy has helped to address the underlying causes of previously untreatable diseases. With three approvals and more than 100 clinical trials in progress, adeno-associated virus (AAV) vectors have emerged as one of the leading gene therapy delivery vehicles [1]. The advancement of viral vector–based gene therapies has led the industry to develop and improve manufacturing scale-up to generate highly pure and potent recombinant adeno-associated virus (rAAV) vectors in HEK293 cells. However, different HEK293 progeny cell lineages vary in gene expression profiles, and adaptation can differentially impact cell metabolism, both of which may result in specific cell-dependent requirements. These differences can pose obstacles to commercial scale-up and often require considerable cell-specific optimization of media [2].

These challenges have driven the need for rAAV vector manufacturers to rapidly identify and optimize a medium specific for the different HEK293 cells and transfection processes. The Gibco™ Viral Vector HEK Media Panel, with five serum-free, chemically defined media, was developed to support rAAV vector production in HEK293 cells by helper-free triple transfection of plasmid DNA. In addition, the Viral Vector HEK Media Panel has the potential to increase viral titers independent of the manufacturing process or cell lineage. As Table 1 shows, the panel design incorporates diverse concentrations of key nutritional components to enable effective and rapid screening for improved media performance with HEK293 suspension cell lines commonly used in rAAV vector production.

Using the Viral Vector HEK Media Panel, cell growth and rAAV production were evaluated for an AAV2 serotype with two different HEK293F cell clones and an AAV8 serotype with cells adapted from the HEK293T cell lineage.

**Table 1. Heat map of Viral Vector HEK Media Panel component diversity.**

| Component | Panel medium 1 | Panel medium 2 | Panel medium 3 | Panel medium 4 | Panel medium 5 |
|---|---|---|---|---|---|
| Amino acids | | | | | |
| Vitamins | | | | | |
| Lipids | | | | | |
| Trace metals | | | | | |
| Polyamines | | | | | |

High level Low level

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## Materials and methods

### Cell culture and adaptation

HEK293F: Two internally derived suspension HEK293F clones (designated 293F1 and 293F2) were evaluated for growth and titer production. After the clones were recovered from the banked medium, both were directly adapted to each test and control medium over three passages. Test media included the five formulations from the Viral Vector HEK Media Panel, identified as panel media 1–5, and Gibco™ FreeStyle™ F17 Expression Medium (Cat. No. A1383501) was the control. Media for the 293F1 and 293F2 cells were supplemented with 8 mM and 4mM GlutaMAX Supplement (Cat. No.35050061), respectively. All of the cultures were seeded at 0.6 x $10^6$ or 0.3 x $10^6$ cells/mL every 3 or 4 days, respectively. Cells were counted using a Vi-CELL™ XR Cell Analyzer (Beckman Coulter).

HEK293T: Cells derived from adherent serum-banked HEK293T cells (ATCC, CRL-3216) were adapted to suspension in a serum-free medium. After recovery from the banked medium, the cells were sequentially adapted to the test or control medium. Test media were medium 1 and medium 5 of the Viral Vector HEK Media Panel, formulations that contain either low levels (panel 1) or high levels (panel 5) of key nutrients. FreeStyle F17 Expression Medium was the control medium. The Viral Vector HEK Media Panel and FreeStyle F17 Expression Medium were supplemented with 8 mM and 4 mM GlutaMAX Supplement, respectively. Subculturing of cells was performed twice a week with seeding at 0.35 x $10^6$ viable cells/mL for a 3-day or 4-day culture in the appropriate test medium in shake flasks. Cells were counted using a Corning™ Cell Counter.

### Transfection

HEK293F: Shake flask cultures were diluted to a density of 3 x $10^6$ cells/mL and transfected with a total of 1.5 µg/mL of plasmid DNA using PEIpro™ transfection reagent (Polyplus). The 293F1 cells were transfected at a 1:1 (w/w) DNA:PEI ratio for panel media 1, 3, 4, and 5, and a 1:2 DNA:PEI ratio for panel medium 2 and the FreeStyle F17 Expression Medium. The 293F2 cells were transfected at 1:1 for all five formulations of the Viral Vector HEK Media Panel and the FreeStyle F17 Expression Medium. The plasmid ratios (w/w) for pAAV-GFP, pRC2, and pHelper (CellBio Labs) were 1:3.03:1.44 for 293F1 cells, and 1:3:1 for 293F2 cells. Cultures were fed glucose up to a final concentration of 6 g/L at 24 hours post-transfection and harvested 72 hours post-transfection. The percent GFP-positive transfection efficiency was quantified by flow cytometry. Data reflect three experiments performed in triplicate.

HEK293T: The plasmid DNA was transfected using PEIpro transfection reagent in biological triplicate shake flasks. One day prior to transfection, cells were seeded at 1.4 x $10^6$ cells/mL. At transfection, cells were inoculated at a density of 2 x $10^6$ cells/mL and transfected with 1 µg of plasmid DNA per $10^6$ viable cells at a 1:1.5 (w/w) DNA:PEI ratio. The plasmid ratio for pHelper (Agilent), pAAV2/8, and pITR-eGFP was 1:1:1 by mass (1:1.6:1.7 molar ratio). Cells were harvested 72 hours post-transfection and percent GFP-positive transfection efficiency was quantified by flow cytometry.

### Viral genome (VG) quantitation by qPCR

HEK293F: Harvested 293F1 and 293F2 cells were lysed and diluted in Invitrogen™ DNase I Buffer. Samples were treated with Thermo Scientific™ Exonuclease I (Cat. No. EN0582) and Invitrogen™ DNase I (Cat. No. 18047019), followed by incubation with Invitrogen™ Proteinase K (Cat. No. AM2548). The AAV2 VG titer was quantified by qPCR using an Applied Biosystems™ TaqMan® Assay (Cat. No. 4332079) targeting GFP. Linearized pAAV-GFP was used to generate the standard curve.

HEK293T: AAV8 was harvested after cell recovery and lysis. Prior to DNase treatment, the lysate was treated with Benzonase™ Endonuclease (MilliporeSigma). The extracts were then treated with DNase, and viral DNA was purified using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH). The VGs were quantified by qPCR using a primer–probe set specific for the *egfp* gene. The pITR-eGFP plasmid was linearized and used to generate the standard curve for quantification.

### Total particles (TP) quantitation by ELISA

HEK293T: The AAV8 fraction particles were quantified using the AAV8 Xpress ELISA assay (PROGEN Biotechnik GmbH). This sandwich ELISA recognizes a specific surface epitope on the assembled capsid via a conformational change that is not present on unassembled capsid proteins. The TP was calculated using a 4-parameter logistic (4PL) regression. The percentage of full capsids (vs. empty capsids) was calculated by dividing the calculated TP by the VG titer.

Note: HEK293T growth and AAV8 productivity evaluations of the Viral Vector HEK Media Panel were conducted by the Institute of Experimental Biology and Technology (iBET, Portugal).

## Results

### HEK293F cell growth and AAV2 productivity

Prior to transfection, the population doubling times for the 293F1 and 293F2 cells were determined to evaluate and compare growth obtained using the Viral Vector HEK Media Panel and the control, FreeStyle F17 Expression Medium. The 293F1 and 293F2 clones demonstrated comparable average doubling times with all five media formulations, relative to FreeStyle F17 Expression Medium (Figure 1). These results indicated the Viral Vector HEK Media Panel formulations did not significantly alter cell growth and would support sufficient growth for productive AAV2 transfection.

The HEK293F VG results revealed that the 293F2 clone produced higher overall average titers than did the 293F1 clone, with all media, as shown in Figure 2. However, both clones produced the highest average VG titers with panel media 4 and 5, compared to FreeStyle F17 Expression Medium, with the 293F1 cells demonstrating 10-fold higher titers and 293F2 cells demonstrating 2-fold higher titers (Figure 2A and 2B).

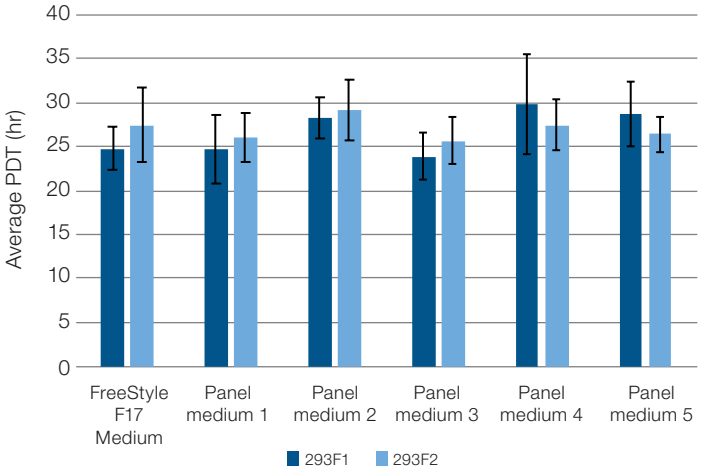


**Figure 1. HEK293F population doubling time.** The 293F1 and 293F2 cell clones had comparable average population doubling times (PDT) in the five Viral Vector HEK Media Panel formulations and FreeStyle F17 Expression Medium.

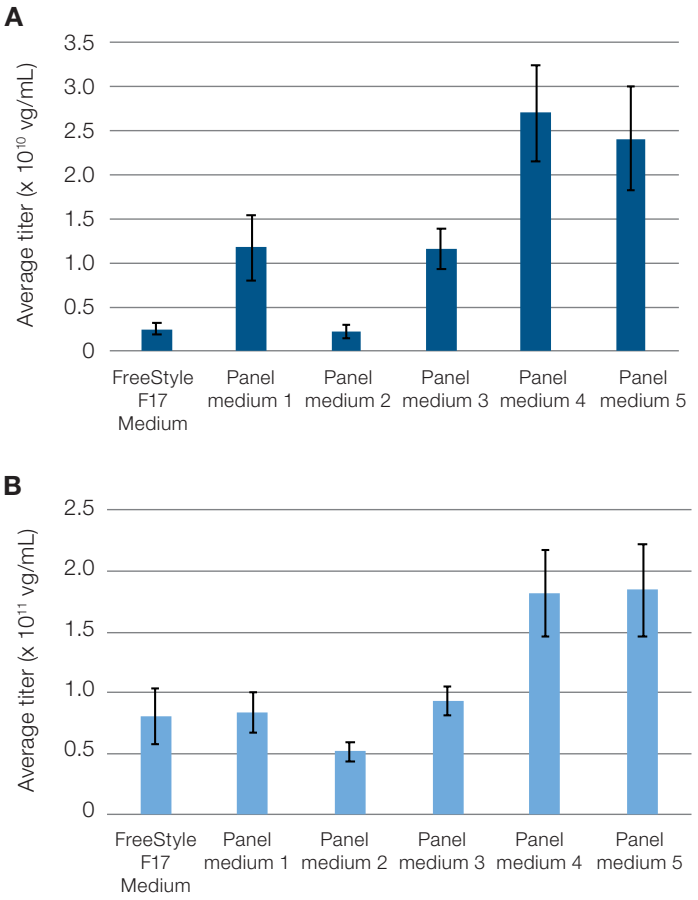


**Figure 2. HEK293F AAV2 viral genome titers. (A)** The 293F1 cells produced 10-fold higher average AAV2 titers with panel media 4 and 5, compared to with FreeStyle F17 Expression Medium. **(B)** The 293F2 cells demonstrated 2-fold higher average titers with panel media 4 and 5, compared to with FreeStyle F17 Expression Medium. (Data reflect three experiments performed in triplicate.)

### HEK293T cell growth and AAV8 productivity

The HEK293T population doubling time in panel media 1 and 5 was comparable to that in the control medium, FreeStyle F17 Expression Medium (23 ± 1 hour) (data not shown), again suggesting that the panel media formulations did not negatively impact cell growth and would support sufficient growth for productive transfection.

The HEK293T AAV8 viral genome titer results were comparable with panel media 1 and 5, compared to FreeStyle F17 Expression Medium (Figure 3A). Determination of full capsids revealed that Viral Vector HEK Media Panel 1 produced an average of 76% full capsids, compared to 51% and 40% with the control medium and panel medium 5, respectively (Figure 3B).

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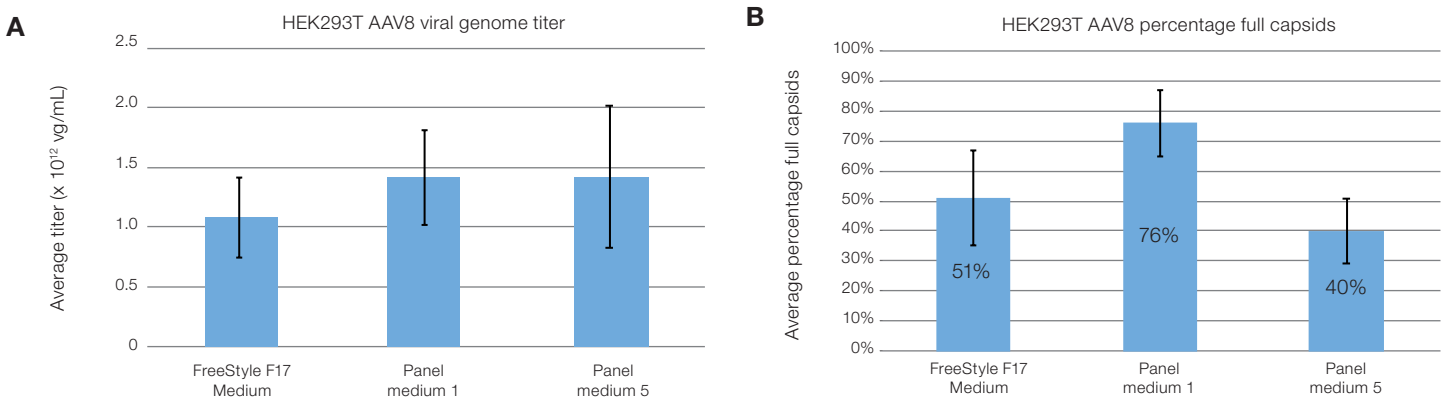


**Figure 3. HEK293T AAV8 viral genome titer and percentage full capsids. (A)** Panel media 1 and 5 yielded comparable average titers when evaluated with FreeStyle F17 Expression Medium. **(B)** Analysis of the average percentage of full capsids showed that panel medium 1 produced 76% full capsids compared to 51% with FreeStyle F17 Expression Medium and 40% with panel medium 5. (Testing was conducted in biological triplicate.)

## Conclusions

AAV manufacturers need to rapidly identify candidate media formulations that can be adapted to their HEK293 cell lines, various AAV serotypes, and transfection processes. Regardless of the HEK293 lineage or clones tested, the Viral Vector HEK Media Panel formulations did not significantly alter cell growth compared to the control, FreeStyle F17 Expression Medium.

Evaluations with the 293F1 and 293F2 clones showed the media panel yielding differential clone-dependent titer responses, respectively demonstrating 10-fold and 2-fold titer increases for panel media 4 and 5 compared to the control, FreeStyle F17 Expression Medium. These findings suggest the potential for further evaluation and optimization of panel media 4 and 5 for the HEK293F cells.

Results with the HEK293T cells demonstrated AAV titer production with panel 1 and 5 was comparable to FreeStyle F17 Expression Medium. In addition to titer production, the percentage of full capsids was evaluated to assess the quality of the AAV product. The evaluation of full versus empty capsids is often considered because empty capsids are a manufacturing impurity that can affect the efficacy and safety of the AAV vector products [3]. The results of this testing revealed an average of 76% full capsids with panel medium 1, compared to 51% and 40% with FreeStyle F17 Expression Medium and panel medium 5, respectively. These results suggest the potential for further analysis and optimization of panel medium 1 for the HEK293T cells.

The Viral Vector HEK Media Panel has demonstrated the potential to address production challenges by enabling rapid screening of media candidates that support increased titers and higher-quality AAV production with diverse HEK293 cell lines. With the rapid changes in AAV manufacturing platforms, the media panel is also poised to enable further process development through formulation optimization.

### References


1. Wagner HJ, Weber W, Fussenegger M (2021) Synthetic biology: emerging concepts to design and advance adeno-associated viral vectors for gene therapy. *Adv Sci* 8:2004018. doi: 10.1002/advs.202004018
2. Grieger JC, Soltys SM, Samulski RJ (2016) Production of recombinant adeno-associated virus vectors using suspension HEK293 cells and continuous harvest of vector from the culture media for GMP FIX and FLT1 clinical vector. *Mol Ther* 24(2):287–297. doi: 10.1038/mt.2015.187
3. Gimpel AL, Katsikis G, Sha S et al. (2021) Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies. *Mol Ther Methods Clin Dev* 20:740–754. doi: 10.1016/j.omtm.2021.02.010


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