FASTFACTS

Addressing HEK293 cell lineage and AAV diversity with media panel

Jennifer Schieber, Senior R&D Scientist, Thermo Fisher Scientific

Adeno-associated virus (AAV) has become an attractive vector for gene therapy; however, low titer yield often limits its viability as a therapeutic. Here, we highlight how a media panel supports AAV production by helper-free triple transfection using HEK293 cells with increased viral titers agnostic of manufacturing processes or cell lineage.

Cell & Gene Therapy Insights 2021; 7(9), 933 • DOI: 10.18609/cgti.2021.124

When it comes to HEK293 cells, each cell line is unique. Since the original HEK293 cells were derived in 1973, the cell line has been repeatedly modified by transfection and adapted to grow in a variety of conditions. Therefore, it's not surprising that HEK293 cell lines demonstrate a significant diversity in genomic and proteomic profiles and nutritional needs. To address this, Thermo Fisher Scientific has developed an approach for basal media screening that increases the chance of finding a great match for a specific HEK293 line and improving performance.

Using principal component analysis, over 60 potential candidates were narrowed down to five formulations, which provide a broad range of different nutrient concentrations and have very specific raw materials **(Figure 1)**.

We tested the compatibility of the media panel with different transfection methods and found that the five panel formulations perform very differently depending on the HEK293 cell line **(Figure 2)**. Response to base media and titers are highly dependent on the HEK293 cell line/lineage/population used. Depending on your cell line, a screen may yield higher results or be a starting point for optimization.

Figure 1. Media panel heat map.

Components	HEK panel medium 1	HEK panel medium 2	HEK panel medium 3	HEK panel medium 4	HEK panel medium 5
Amino acids					
Vitamins					
Lipids					
Trace metals					
Polyamines					

High level — Low level

CASE STUDY 1: EVALUATION OF DIFFERENT SEROTYPES

The viral vector HEK media panel was evaluated in a single cell line and multiple AAV serotypes. For this experiment, only the top three formulations were considered, and f17 was used as the control media.

The panel formulations yielded similar genomic titer, cell growth, and transfection efficiency compared to F17. However, when harvested fractions were analyzed separately for intracellular and secreted AAVs, some differences did emerge. Looking at serotypes in which AAV was mostly secreted (AAV8 and AAV9), there was an apparent correlation between using media panel 4 and an increased amount of AAV in the extracellular fraction **(Figure 3)** – a finding that warrants further investigation.

Figure 2. Potential to increase titer with Viral Vector HEK Media Panel. All titers normalized to FreeStyle F17 Expression Medium. Titers range from 109 to 1011 vg/mL depending on the cell line and process.

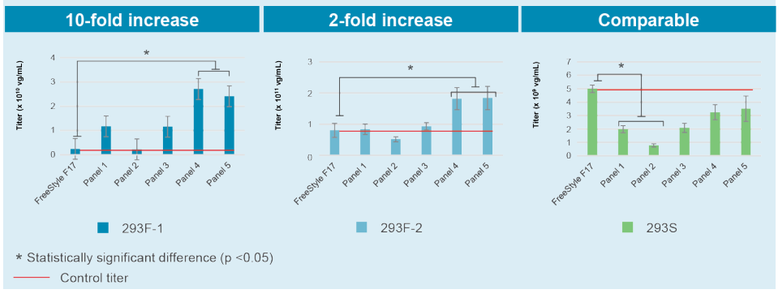


Figure 3. Increased yield for secreted serotypes. Apparent increased extracellular vg/mL in Panel 4 on AAV8 and AAV9. At scale, the AAV8 process has been qualified without a lysis step at harvest to limit contaminants. Panel 4 was selected to be validated in future bioreactor runs for AAV8 production.

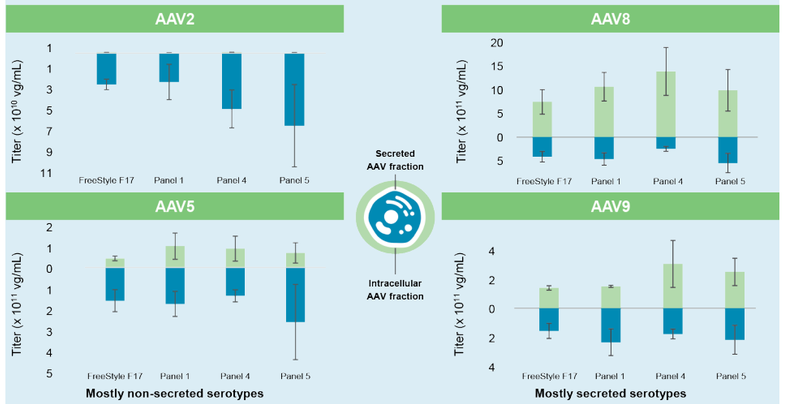
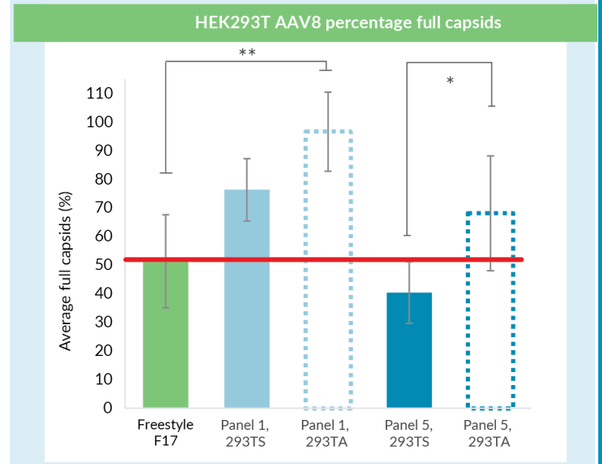


Figure 4. HEK293T AAV8 percentage full capsids. Percentage of full capsids determined by qPCR and ELISA analysis. All experiments were performed on AAV8 with comparable total yield (vg/mL).



CASE STUDY 2: IMPACT OF ADAPTATION STRATEGY

The Viral vector HEK media panel was also evaluated for use in adapting cells from adherence to suspension. Only panels 1 and 5 were tested as they have the most differentiated level of nutrients.

Once cells were adapted, triplicate biological replicates were run to assess if the adaptation strategy had an impact on titers or particle quality after performing AAV8 triple transfections. No significant difference was seen in average genomic titer between F17, panel media 1, or panel media 5.

However, looking at the empty:full ratio, we saw an apparent increase in % full capsids when cells were adapted directly into the panel media (293TA) compared to having been first adapted to the control medium (293TS) **(Figure 4)**.