# HMSP Growth Media Significantly Improve HEK293 Growth and Production of AAV2

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## INTRODUCTION

Human embryonic kidney (HEK293) cells are the most commonly used cell line to produce viral vectors for gene therapy. However, the complexity of their production and purification as well as the therapeutic demand for high dosage, make viral vectors very expensive to produce.

Choosing the proper media in a scale-down model for each cell line and process, improves the yield and lowers the production costs.

HEK Media Survey Panel (HMSP) is composed of six chemically defined media that enable users to quickly and efficiently survey a curated panel of high-quality, scalable HEK media for application development.

Backed by more than 50 years of cell culture media expertise, FUJIFILM Irvine Scientific offers this panel to help manufacturers in cell and gene therapy, and bioproduction discover the optimal media for increased titers, quality, and commercial readiness.

In this case study, we evaluated HMSP in comparison with another commercially available HEK panel from Supplier A, with regard to cell growth and adeno-associated virus serotype 2 (AAV2) production.


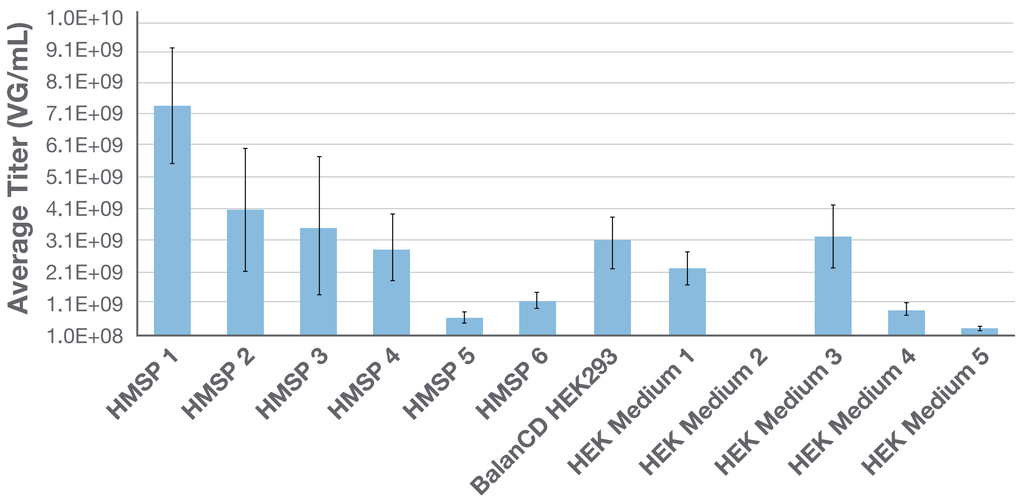


**Figure 1. AAV2 titer of HMSP and HEK media panel (Supplier A).** The viral genome (VG) titer was measured 72 hours post transfection. Cell extracts were released after three rounds of freeze-thaw cycles, treated with DNase and lysis buffer, and viral genome copy measured using AAVpro Titration kit for real time PCR version 2 (TakaraBio).

## MATERIAL AND METHODS

- **Cell stock:** Expi293 (HEK293F, Thermo Fisher Scientific)
- **Culture vessel:** 125 mL Corning shake flask with vent cap
- **Seeding density for growth phase:** 3x10$^5$ cells/mL
- **Seeding density for transfection phase:** 1x10$^6$ cells/mL
- **Transfection agent:** Polyethylenimine (PEIpro, PolyPlus)
- 3 plasmids for AAV2 (TakaraBio)
- **DNA:** PEI ratio at 1:2
- Total amount of 1.5 µg/mL

## SUMMARY

- A panel of six chemically defined, animal component-free media known as HEK293 Media Survey Panel (HMSP) was specifically developed to support viral vector production in HEK293 cell lines.
- Optimal media can be selected by testing the panel based on the cell line, viral vector, plasmids, transfection reagent, and the process.
- Data collected on Expi293 cell line indicated that the panel contains some media that outperform commercially available HEK media panel as well as our current off-the-shelf BalanCD HEK293 in cell growth health indices (VCD and viability) and the final AAV2 production yield.

## RESULTS

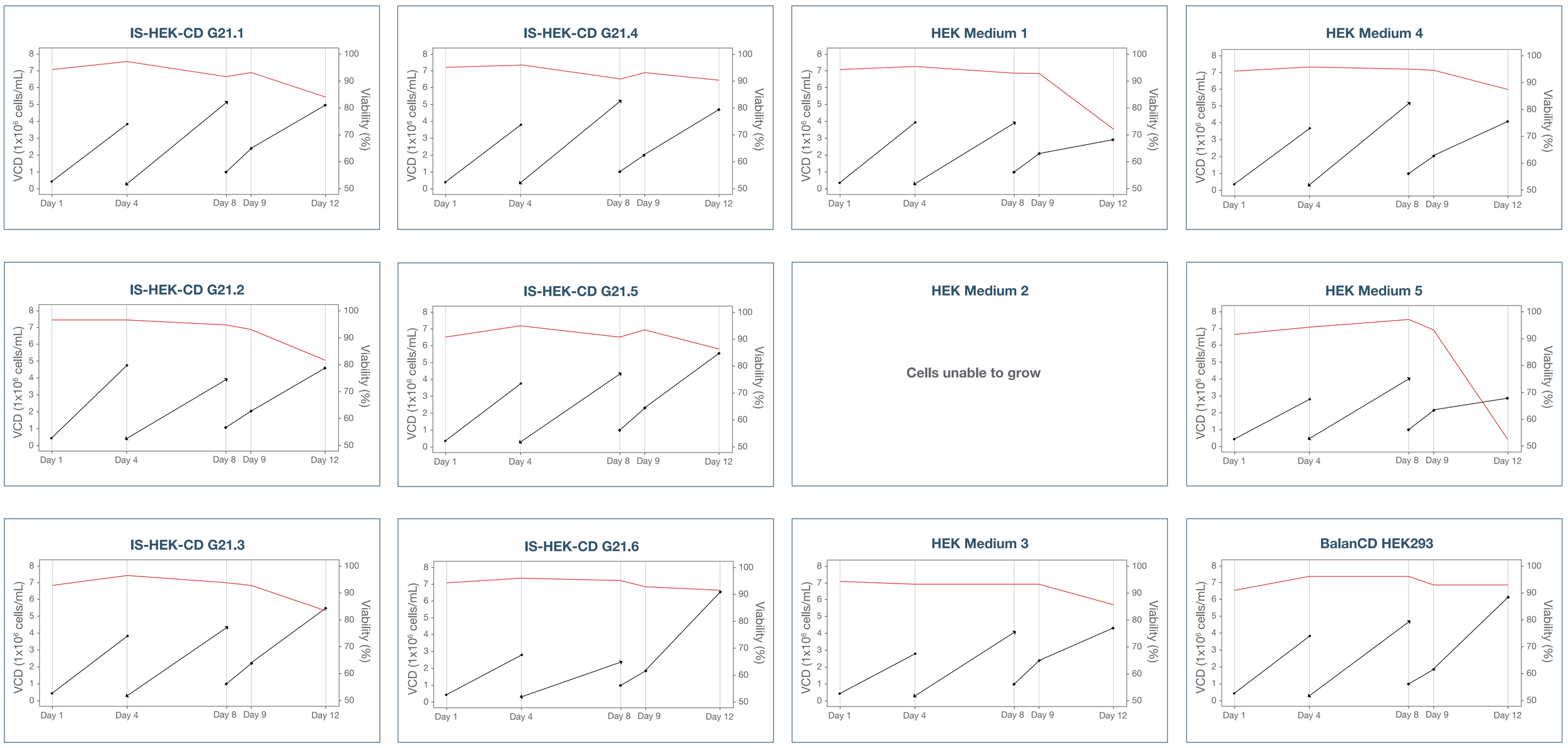


**Figure 2. Cell growth pre- and post-transfection with AAV plasmids; cells cultured in HMSP (IS-HEK-CD) and HEK panel from Supplier A (HEK Media) as well as BalanCD HEK293 medium as a control**. Expi293 Cells (Thermo Fisher Scientific) adapted and banked in BalanCD HEK293 medium (FUJIFILM Irvine Scientific), thawed in BalanCD HEK293 and passaged in the corresponding media for two passages and seeded at 1X10$^6$ cells, 24 hours before transfection. Cells were transfected using triple transfection with Takara AAV2 plasmids (GOI: ZsGreen, RC, and Helper), 1.5 µg/mL DNA mixed with PEIpro (PolyPlus) at 1:2. Cell growth and viability was monitored before and after each subculture, post transfection, and at the harvest time. Viable cell density (black line) and percent viability (red line) were measured using ViCell cell counting instrument.