# Chemically Defined BalanCD HEK293 Viral Feed Boosts Viral Vector Production

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## BACKGROUND

Various strategies have been adopted to boost viral vector production in HEK293 cells, the most commonly used host cell line for producing viral vectors in cell and gene therapy as well as vaccine development. Approaches include optimization of basal/growth media, transfection reagents or enhancers, and modified cell lines. While such approaches often require costly process optimization in well-established viral vector production systems, we developed BalanCD HEK293 Viral Feed, a chemically defined feed medium, to boost viral vector production in suspension cell cultures. BalanCD HEK293 Viral Feed is compatible with a wide range of basal media, transfection reagents, and cell lines. For standard batch production of viral vectors in HEK293 cells, it can be easily converted to fed-batch with minimal disruption to the production workflow. BalanCD HEK293 Viral Feed is applied at 12% (v/v), 24 hours post-transfection to boost viral vector production. It improves AAV (Adeno-Associated Virus) titer in various HEK293 cell lineages by 2-fold to 8-fold, without any adverse effect on cell growth and viability. The enhanced production, 3-fold to 6-fold of viral titer, has been shown for producing different serotypes of AAV, such as AAV2, AAV5, and AAV9. The improvement upon feed is also scalable in bioreactors.

## MATERIAL AND METHODS

**Seeding density for transfection:** $1\times10^6$ cells/mL 24 hours prior to transfection for PEI transfection; $2.5\times10^6$ cells/mL at the time of transfection for AAV-MAX transfection

**Transfection reagent:** Polyethylenimine (PEIpro, PolyPlus) and AAV-MAX Transfection Reagent from AAV-MAX Helper-Free AAV Production System (Thermo Fisher Scientific)

**DNA:** triple transfection with AAV2, AAV5, and AAV9 plasmids (GOI/Gene of interest: ZsGreen/GFP, RC: Rep-Cap, and Helper plasmids) with 1:1:1 molar ratio and a viral vector concentration of 1 or 1.5 µg/mL cell culture

**DNA:PEI** ratio at 1:2; AAV-MAX Transfection Reagent: 6 µL per mL of cell culture

Cells were transfected using the complex solution of DNA and PEI or AAV-MAX Transfection Reagent in cell growth medium per recommended protocol. Cells were fed with BalanCD HEK293 Viral Feed at 24 hours post-transfection using 12% v/v of the culture. The cells were harvested 72 hours post-transfection. The cell lysis was used for viral genome and capsid titer assays.

## RESULTS

### *Demonstrated in Three Independent HEK293 Cell Lines*

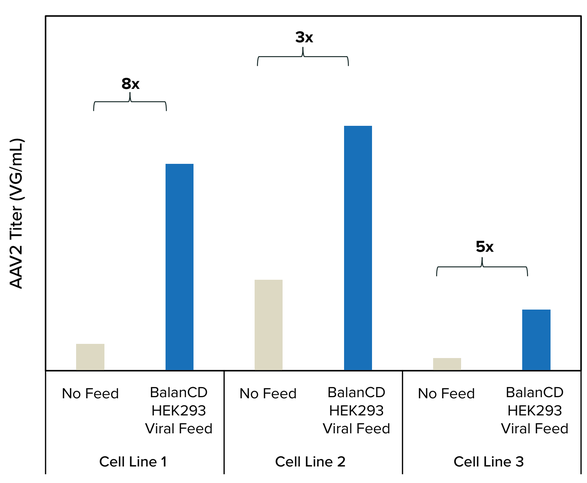


**Figure 1. BalanCD HEK293 Viral Feed boosts AAV2 production in various HEK293 cell lines.** HEK293 cells (3 cell lines) were cultured in triplicate using BalanCD HEK293 medium and supplemented with 12% (v/v) BalanCD HEK293 Viral Feed at 24 hours post-transfection or no feed control. The cells were harvested 72 hours post-transfection. The AAV2 titer in each cell culture was quantified by qPCR using AAV real-time PCR titration kit (TakaraBio) for Cell Line 1 and Cell Line 2, and digital PCR (dPCR, QIAcuity) for Cell Line 3. In all cases, ITR primers were used. The error bars stand for standard deviations of triplicates of cell cultures under each culture condition with triplicates of qPCR or dPCR for each cell culture. Fold titer improvement over no feed control is shown above each condition set.

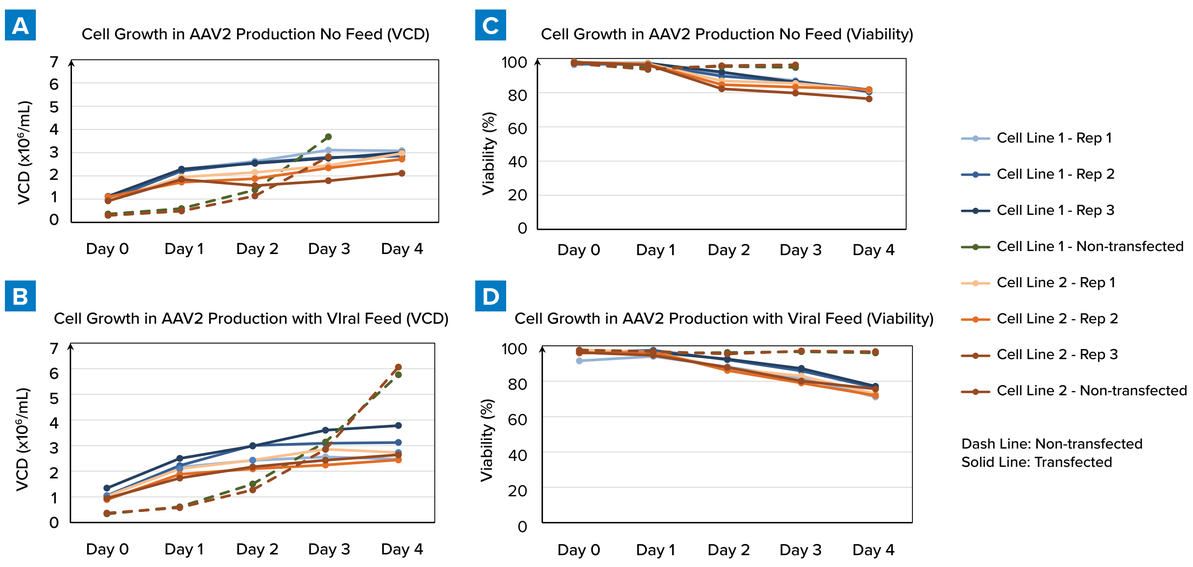


**Figure 2. BalanCD HEK293 Viral Feed shows no adverse effect on HEK293 cell growth and viability.** VCD (**A** and **B**) and viability (**C** and **D**) of Cell Line 1 and Cell Line 2 under no feed (**A** and **C**) or fed with BalanCD HEK293 Viral Feed (**B** and **D**) were measured on Day 0 to Day 4, with transfection on Day 1. The dash lines represents the control condition (non-transfected cells). VCD and viability were not impacted by the addition of feed.

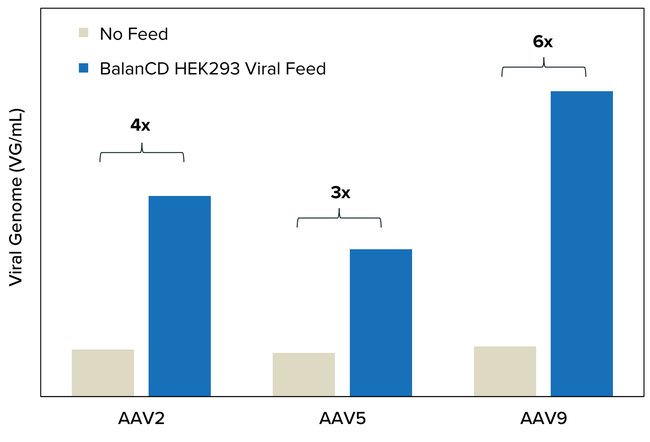


**Figure 3. BalanCD HEK293 Viral Feed increases AAV titer in different AAV serotypes.** HEK293 cells were cultured in 30 mL of BalanCD HEK293 medium and triple transfected with viral vectors expressing different AAV serotypes at a 1 µg/mL viral vector concentration. BalanCD HEK293 Viral Feed (12% v/v) was applied at 24 hours post-transfection. The cells were harvested 72 hours post-transfection. AAV titer was measured by digital droplet PCR (ddPCR, Biorad). Fold titer improvement over no feed is shown for each serotype.

### *Demonstrated in Various Basal Growth Media*

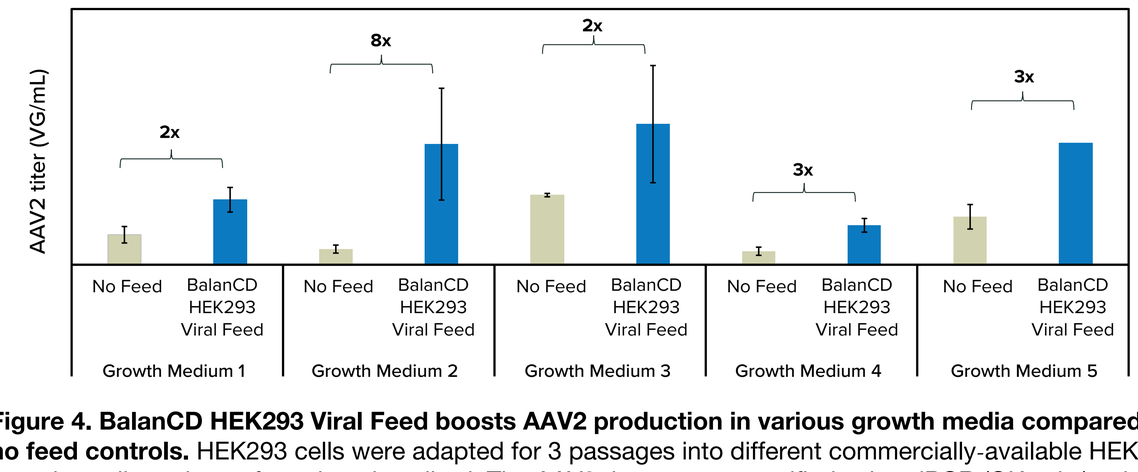


**Figure 4. BalanCD HEK293 Viral Feed boosts AAV2 production in various growth media compared to no feed controls.** HEK293 cells were adapted for 3 passages into different commercially-available HEK293 growth media and transfected as described. The AAV2 titers were quantified using dPCR (QIAcuity) using ITR primers. The error bars stand for standard deviations of triplicates of cell cultures under each culture condition with triplicates of dPCR for each culture set. Fold titer improvement over no feed control is shown above each condition set.

### *Demonstrated in Lipid-based Transfection*

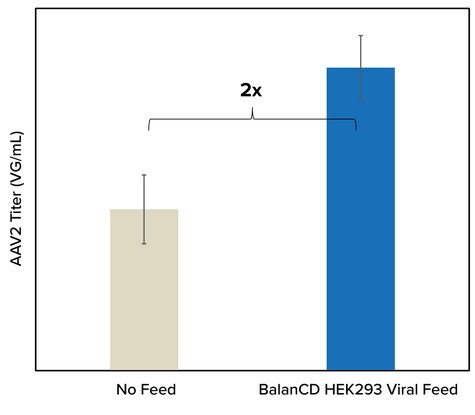


**Figure 5. BalanCD HEK293 Viral Feed is compatible with lipid-based transfection.** AAV2 were produced using AAV-MAX Helper-Free AAV Production System. Cells were grown in BalanCD HEK293 medium and transfected using AAV-MAX transfection kit per manufacturer's protocol. BalanCD HEK293 Viral Feed was added 24 hours post-transfection, at 12% v/v. Viral genome titer was measured by dPCR (QIAcuity) using ITR primers. The error bars stand for standard deviations of 2 replicates of cell cultures under each culture condition and triplicates of dPCR for each cell culture. Fold titer improvement over no feed control is shown.

### *Demonstrated in Bioreactor at 2 L and 10 L Scales*

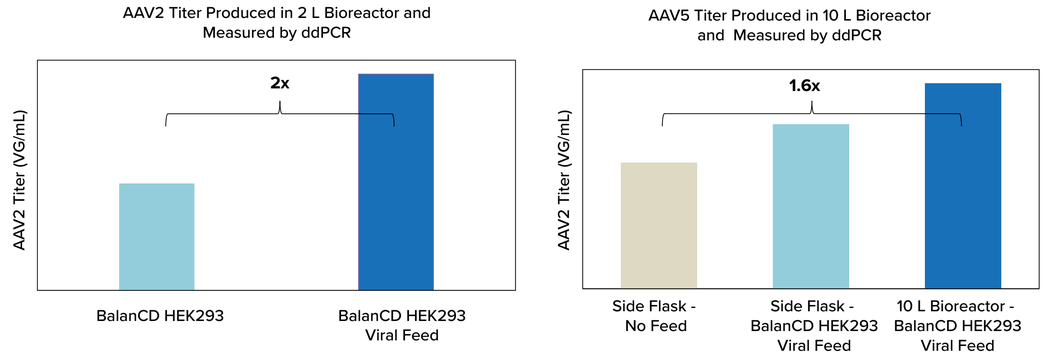


**Figure 6. BalanCD HEK293 Viral Feed performance in bioreactors. Verification of the BalanCD HEK293 Viral Feed performance of improving the production of AAV2 in 2 L and AAV5 in 10 L scale bioreactor.** HEK293 suspension cells were seeded at density of $8\times10^5$ cells/mL in XDR2 for AAV2 and XDR10 for AAV5. BalanCD HEK293 Viral Feed was applied 12% (v/v), at 24 hours post-transfection. The cells were harvested 72 hours post-transfection. The AAV2 titer in each vessel was quantified using ddPCR in triplicate. The average of ddPCR replicates was plotted. Fold titer improvement over the control is shown**.**

### *Demonstrated in Adherent HEK293T cells*

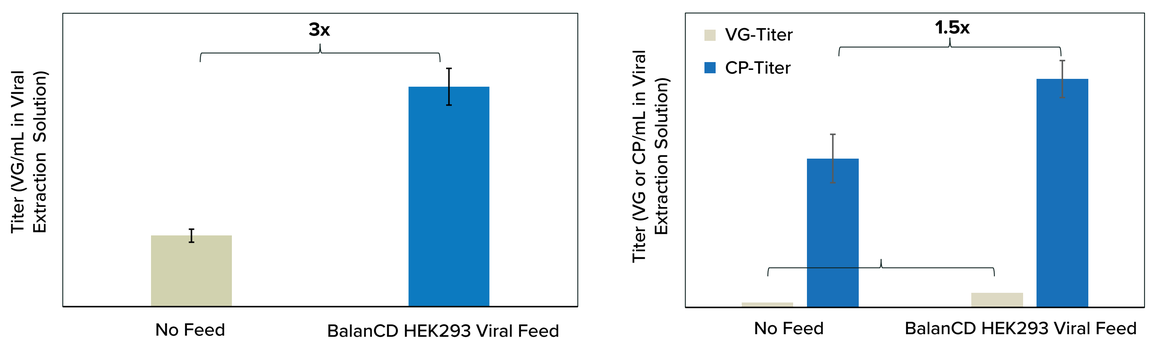


**Figure 7. BalanCD HEK293 Viral Feed boosts AAV2 production in adherent HEK293T cells.** HEK293T cells were seeded in 6-well plates using complete culture medium (DMEM + 10% FBS) 24 hours before transfection with triple AAV2 plasmids (TakaraBio). The complete culture medium was replaced with serum-free DMEM at the time of transfection. Cells were incubated with DNA-PEI complex in serum-free medium for 4 hours until the complete culture medium was added into each well to 3 mL/well. BalanCD HEK293 Viral Feed (12% v/v) was applied at 24 hours post-transfection. The cells were harvested 48 hours post-transfection. The AAV2 titer in each well was quantified using dPCR in triplicate. The error bars represent triplicates of cell cultures under each culture condition with triplicates of dPCR for each cell culture. Fold titer improvement over no feed control is shown.

## SUMMARY

We developed a chemically defined, animal component-free feed supplement that increases AAV titers by 2-fold to 8-fold and improves viral vector production. Supporting both suspension and adherent HEK293 cell lines, BalanCD HEK293 Viral Feed is compatible with a wide range of basal media and transfection reagents, and it is adaptable for use in multiple AAV serotypes. Through this study, we demonstrated a simplified, scalable process to convert from batch to fed-batch production of viral vectors for cell and gene therapy.