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White Paper

A Novel Approach to Improving AAV Titers Using the Sf-RVN® Platform

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Summary

Adeno-associated viruses (AAVs) are pivotal in viral vector production, yet challenges like low productivity, high production costs, and manufacturing complexity hinder large-scale GMP manufacturing. To address the rising demand for higher AAV titers, we evaluated Cellvento® ModiFeed Prime feed as a supplement for enhancing AAV production in Sf9 cell lines.

Supplementing the Sf-RVN® cells with Cellvento® ModiFeed Prime feed following baculovirus infection led to significant increases in AAV titer. Addition of the supplement also resulted in improved AAV quality with a higher proportion of full capsids as compared to non-supplemented cultures. Although the magnitude of improvement was cell line and virus strain dependent, the benefits of Cellvento® ModiFeed Prime feed supplements were observed in several AAV or virus-like particle producing platforms, suggesting broad benefits of this complex supplement.

As demand for AAV-based therapies rises, optimizing production processes is essential. By utilizing existing technologies, tailored platforms, and optimized formulations, titers and productivity can be enhanced.

Background

AAVs have become a cornerstone of viral vector production. However, while AAVs offer immense potential for gene therapy, the challenges associated with their production can hinder large-scale commercialization and delay advancing a viral gene therapy to the clinic or market. Manufacturing challenges include low productivity, a growing demand for higher titers and larger volumes, manufacturing complexity, and high production costs.

In response to the increasing needs for higher AAV titers, we screened the MilliporeSigma portfolio of catalog media and feeds to identify options that might improve the productivity of AAV producing cell lines. This white paper summarizes the results of screening studies that identified Cellvento® ModiFeed Prime feed as a supplement capable of significantly increasing AAV titers in the Sf-RVN® platform.

Sf-RVN® Platform for AAV Production

The Sf-RVN® platform consists of the Sf-RVN® insect cell line and the EX-CELL® CD Insect Cell Medium, which is a chemically defined cell culture medium. The Sf-RVN® cell line is certified to be free from rhabdovirus contamination. Although rhabdovirus is not considered harmful to humans, it was identified by the FDA in 2014 as a contaminant of most Sf9 cell lines. Consequently, manufacturers using Sf9 cell lines must demonstrate sufficient clearance capabilities of rhabdovirus in their production process.

AAV production using the Sf-RVN® cell platform is initiated by infecting cells with one or more baculoviruses; this is known as the baculovirus expression vector system (BEVS) (**Figure 1**).

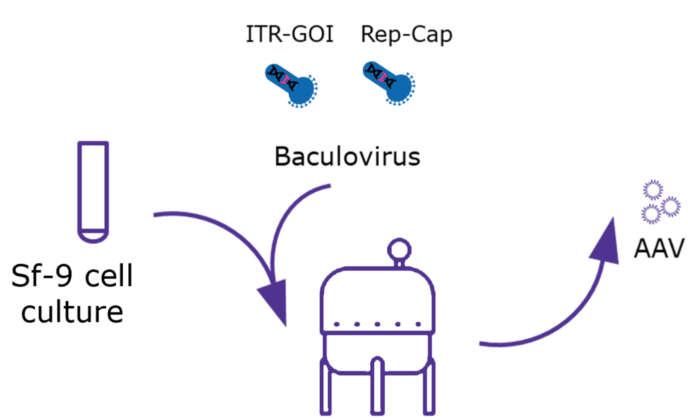

Figure 1
Sf-RVN® Platform for AAV production using BEVS

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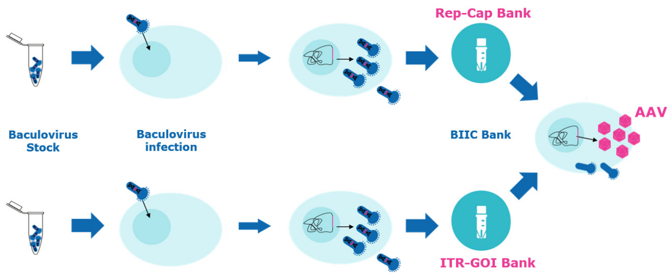


Figure 2
Sf-RVN® Platform for AAV production using BIICs

An alternative option for AAV production is the baculovirus-infected insect cell (BIIC) system where baculovirus infected Sf-RVN® cells are harvested and cryopreserved (**Figure 2**). BIIC banks provide long-term stability and, when needed, aliquots of the bank can be thawed and amplified in cell culture to produce AAV. The Sf-RVN® platform is suitable for AAV production using both BEVS and BIIC approaches.

The first step for AAV production in Sf-RVN® cells is to create the baculovirus. To create the stocks of baculovirus, Sf-RVN® cells are transfected with bacmids, encoding either the RepCap gene or the gene of interest. Following incubation, baculovirus is harvested and stocks titered by plaque assay or similar methods. These baculovirus stocks are then used to produce AAV using a two-BAC system or two-BIIC system.

The Sf-RVN® cells were split to a density of 2×10^6 cells /mL from a healthy exponentially growing culture. Cells were infected on the day of splitting and monitored throughout the production phase. Cell morphology was monitored throughout the duration of cell culture as the Sf-RVN® cells swell from a normal diameter of about 17 µm when uninfected to about 23 µm post infection. Once cell viability drops below 70%, the cell culture material was harvested and lysed with a Deviron® 13-S9 detergent solution, which contained Benzonase® endonuclease. This crude lysate was collected, and AAV titers analyzed via Digital Droplet (dd) PCR, and affinity-purified samples were analyzed for empty/full capsid profile using mass photometry (SamuxMP; Refeyn Ltd).

Optimizing Supplementation: Volume and Timing

The supplementation strategy for the Sf-RVN® cell line was optimized: preliminary studies revealed that the addition of L-glutamine to Cellvento® ModiFeed Prime feed increased the magnitude of AAV titer improvements. As a result, extra L-glutamine was always included with the Cellvento® ModiFeed Prime feed supplement when added to the insect cell culture. **Figure 3** shows the benefits of various levels of Cellvento® ModiFeed Prime feed and 15 g/L L-glutamine supplements to AAV2 titers on the Sf-RVN® cell line when added at either 24 hours post infection (hpi) or at 24 hpi and 48 hpi.

All test conditions with the Sf-RVN® cell line that were supplemented with the Cellvento® ModiFeed Prime feed and L-glutamine yielded between a 60- and 70-fold increase in AAV2 titer. Results show there is flexibility on timing of supplement addition: addition of 5% supplement at both 24 hpi and 48 hpi yielded slightly higher titers than a single supplement at 24 hpi. However, addition of a 5% or 10% supplement at either 24 hpi or 24 hpi and 48 hpi did not significantly improve titer beyond the single supplement at 24 hpi, which offers the added benefit of simplified implementation.

These results show that adding 5–10% Cellvento® ModiFeed Prime feed and L-glutamine to the Sf-RVN® cell line at 24 hpi significantly increases AAV titer.

Sf-RVN® Cell Line Comparison and Competitor Feed Evaluation

The benefits of the Cellvento® ModiFeed Prime feed supplemented with L-glutamine for the Sf-RVN® platform were benchmarked against another commercially available feed supplement, Feed A. Culture conditions included 1 L bioreactors with a 750 mL working volume and a pH setpoint of 6.2, 40% dissolved oxygen, and an agitation rate of 200 rpm with Sf-RVN® cells seeded at a density of 2×10^6 cells/mL. These 1 L bioreactors were infected using the BIIC system:

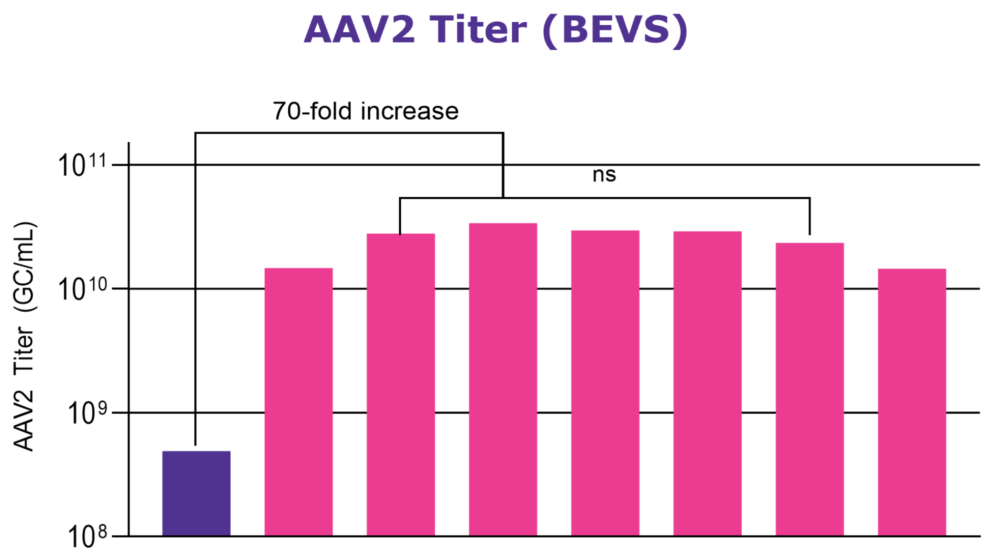
- A BIIC cell bank infected with the RepCap baculovirus was added at a density of one infected cell for every 10,000 non-infected cells (1/10,000).
- A BIIC cell bank infected with the gene of interest was added at a density of one infected cell for every 20,000 non-infected cells (1/20,000).

Cultures were supplemented with Cellvento® ModiFeed Prime feed and L-glutamine or another commercially available feed, “Feed A”: 10% at 24 hpi and 5% at 48 hpi. **Figure 4** (left) shows the AAV2 titers achieved with the various test conditions.

The benefits of Cellvento® ModiFeed Prime feed and L-glutamine to AAV titer in 1 L bioreactors were similar to the increase observed in small-scale evaluations: a 71-fold increase in titer, which was higher than the titer increase observed with Feed A.

The empty/full capsid ratios of the non-fed culture and Cellvento® ModiFeed Prime feed conditions in the 1 L bioreactor studies are shown in **Figure 4** (right). Using Cellvento® ModiFeed Prime as a post-infection feed increased the ratio of full capsids from 25% in the non-fed culture condition to 52% in the supplemented conditions.

These results confirm the benefits of supplementing Sf-RVN® cells with Cellvento® ModiFeed Prime feed and L-glutamine on both AAV titer and AAV capsid quality in 1 L bioreactors.



	Non-Fed	Cellvento® ModiFeed Prime + L-Glutamine						
24hpi		5%	5%	5%	10%	10%	10%	15%
48hpi		-	5%	10%	-	5%	10%	-

10% (v/v) at 24 hpi easy to implement

Figure 3
AAV2 production in the BEVS system with Sf-RVN® when adding Cellvento® ModiFeed Prime + L-glutamine at various volumes and times. The non-fed control is purple and the conditions receiving the post infection feed are shown in pink. (ns = no significant difference)

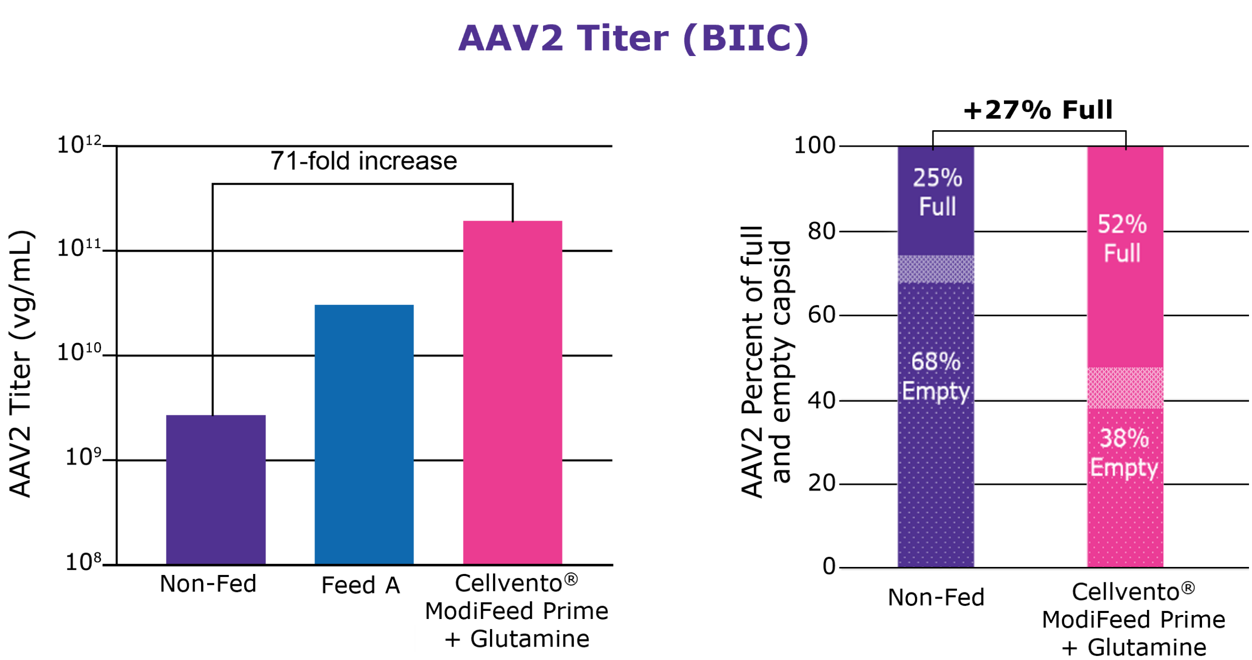


Figure 4
AAV2 titer using the BIIC system; 1 L bioreactor, 10% fed at 24 hpi and 5% at 48 hpi AAV titer (left) and empty/full capsid ratio of supplemented cultures compared to non-fed culture (right)

The benefits of supplementing Sf-RVN® cells with Cellvento® ModiFeed Prime feed and L-glutamine were explored with the Sf-RVN® cell line and another AAV serotype, AAVrh.10. In these studies, 10% v/v Cellvento® ModiFeed Prime feed and L-glutamine were added at 24 hpi with an additional 5% v/v feed at 48 hpi.

Results show a 567-fold increase in AAVrh.10 titer when Cellvento® ModiFeed Prime feed and L-glutamine were supplemented post-infection (**Figure 5**).

Results from another study extend these findings to other cell lines and virus expression systems. **Figure 6** shows the increase in human papillomavirus virus-like particles (VLPs) titer when Sf9 cells were supplemented with 5% v/v Cellvento® ModiFeed Prime feed and L-glutamine at both 24- and 72-hpi. When virus was harvested at 96 hpi, the conditions supplemented with Cellvento® ModiFeed Prime feed showed a two-fold increase in VLP titer as compared to the non-supplemented control.

Overall, these data demonstrate that supplementing Sf-RVN® and Sf9 cells with Cellvento® ModiFeed Prime feed and L-glutamine post-infection increases titer of multiple AAV serotypes and VLPs.

Benefits of Cellvento® ModiFeed Prime feed for AAV production

Cellvento® ModiFeed Prime feed offers several advantages as a post infection agent for maximizing AAV productivity, including:

- **Compacted formulation:** As a compacted dry powder feed, Cellvento® ModiFeed Prime feed provides reduced bulk volume and easy handling through minimized dust formation and improved flowability.
- **Simplified hydration:** The formulation requires fewer steps and shorter mixing times, eliminating the need for pH or osmolality adjustments prior to use.
- **High concentration:** At a concentration of 131 g/L, low percentages of Cellvento® ModiFeed Prime feed are sufficient for supplementation.
- **Reliable supply chain:** A dependable supply chain with double-sourced components supports a robust production process.

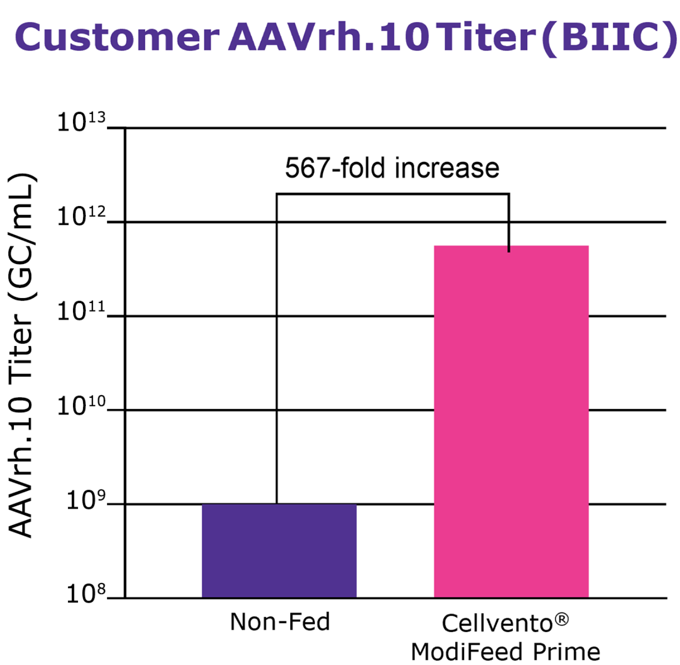


Figure 5
Customer data of AAVrh.10 titer in the BIIC system using a 125 mL shake flask, 10% fed at 24 hpi and 5% fed at 48 hpi

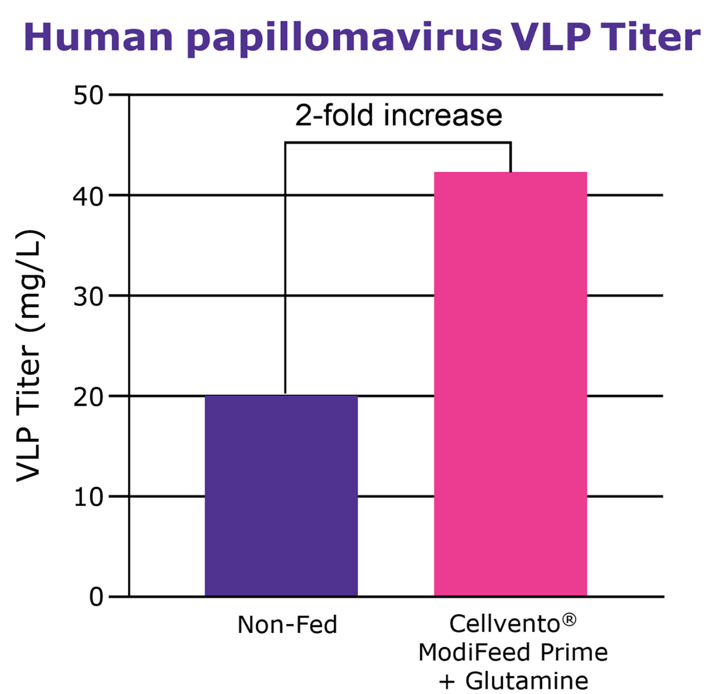


Figure 6
Human papillomavirus VLP titer in the Sf9 cell line adding 5% Cellvento® ModiFeed Prime at 24 hpi and 72 hpi, with harvesting at 96 hpi

Conclusions

The data presented demonstrate that Cellvento® ModiFeed Prime feed and L-glutamine improve AAV production in Sf-RVN® and Sf9 insect cell lines.

- For AAV production using the Sf-RVN® insect cell platform, supplementing cell cultures can be performed 24 hpi or 24 and 48 hpi. There is flexibility in the supplementation regime: supplementing with 10% Cellvento® ModiFeed Prime feed and L-glutamine at 24 hpi provided the same benefits as dual supplements at 24 and 48 hpi.
- Importantly, supplementing cultures with Cellvento® ModiFeed Prime feed and L-glutamine improved the quality of AAV capsids, with a higher proportion of filled capsids in supplemented cultures.
- The benefits of supplementing with Cellvento® ModiFeed Prime feed and L-glutamine are not limited to the Sf-RVN® cell line. Supplementing Sf9 cell cultures post infection improves titers of VLPs and may provide an easy to implement option for improved viral productivity.

These advancements in AAV production methodologies highlight the potential for significant improvements in viral vector manufacturing. As the demand for AAV-based therapies grows, optimizing production processes is crucial to meet future needs. By leveraging existing technologies, tailored production platforms, and optimized formulations, titers and productivity can be significantly increased. The flexibility and adaptability of the Sf-RVN® platforms, along with the benefits of supplementing cell cultures with Cellvento® ModiFeed Prime feed, offer exciting opportunities for further innovation, including the potential for serotype-specific optimizations and the exploration of new applications in gene therapy.

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06/2025