# Virus Yield Improvement for Downstream Processing: Exbumin®, Excipient Recombinant Albumin

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## INTRODUCTION & OBJECTIVES

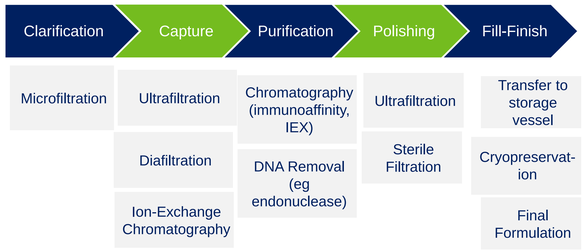
Biological manufacturers face many challenges in downstream processing unit operations of viral based therapeutics that impact the integrity, stability, and potency of their final drug product. Human Serum Albumin (HSA) has been shown to improve yield during common downstream purification unit operations such as tangential flow filtration, depth filtration, chromatography, sterile filtration, and final formulation of viruses and viral vectors. However, the use of serum-derived proteins is discouraged due to supply chain and regulatory concerns as well as concern about potential to introduce adventitious agents.

Here we demonstrate that a recombinant excipient-grade albumin, known as Exbumin®, increases viral yields via virus stabilization during downstream purification unit operations as well as in final formulation while retaining consistency and safety of the final product. Taken together, incorporation of Exbumin® into downstream viral purification protocols can provide benefit for large scale cell/gene therapy and vaccine manufacturing.

Exbumin® Recombinant HSA ("rHSA") has benefits, such as:

- **Recombinant** - improved performance over native blood-sourced HSA
- **Sustainable** - no animal origin components
- **Scalable** – metric ton capacity removes supply chain concerns

*Figure 1. Overview of a typical viral vector/ vaccine downstream manufacturing process[1]*




## CHALLENGES IN DOWNSTREAM PROCESSING UNIT OPERATIONS

Production of viral vectors and viruses is a complex process that requires innovative approaches to meet safety and efficacy requirements, clinical and market demands, and cost of goods targets. Preparing stable viral vectors, preventing their degradation during manufacturing, handling, storage, and maintaining their long-term stability and efficacy are notable obstacles for the manufacturer.[2] More specifically, common challenges include the following:

### Surface Adsorption & Non-Specific Binding

- Viral vectors may be lost or denatured after nonspecifically binding surfaces during production, purification, and filtration. Non-specific adsorption and unfolding may also occur during final product formulation

### Chemical & Thermal Instability

- Viral vectors and viruses are temperature-sensitive and can lose stability when exposed to high temperatures.
- Repeated freeze-thaw cycles can lead to denaturation of viral proteins, resulting in loss of infectivity.
- Variation between vector types creates unique environmental conditions that are optimal for processing and storage.[2]
- Currently, most of the clinical and commercial AAV/LV products are stored frozen because they do not have long-term storage stability under refrigerated conditions. For example, Luxturna and Zolgensma are stored frozen, and they have only one year of shelf-life under frozen conditions.[2]

### Viral Aggregation

- Virus particles can aggregate or lose functionality as a result of shear stress during the filtration process.
- Factors such as temperature, impurities, type and concentration of denaturant, pH, ionic strength, refolding catalysts, hydrophobic interactions, and miscellaneous additives have been found to affect protein aggregation during refolding.

### Degradation

- Significant damage to integrity of AAV/LV can be caused by the use of sub-optimal buffer conditions.
- The presence of proteases and nucleases in culture medium or processing buffers can cause enzymatic degradation of virus. The enzymes can degrade the capsid or the viral genome.

### Shear Stress

- Virus is often forced through high pressure conditions during purification, and concentration steps. These forces create high shear force environments as the vector/virus is processed. Mechanical agitation, fluid turbulence, and shear forces from pumps/filters can lead to a loss of infectivity, reduced efficacy, and reduce final virus yield.

## THE USE OF ALBUMIN TO REDUCE VIRUS LOSS AND IMPROVE TITERS

Exbumin® has been utilized to improve overall titer yield in common downstream unit operations, including: TFF, Depth filtration, chromatography, sterile filtration, and final formulation. While the exact mechanisms of action are not yet fully understood, several hypotheses exist:

- **Reduction of Loss Due to Adsorption** due to the presence of albumin in solution and as a surface pre-treatment
- **Increased Thermal Stability and Shelf-Life** of final drug product
- **Reduction of Aggregation** where albumin in virus and vector preparations supports homogeneity to reduce aggregation
- **Stabilization** due to albumin interactions with vectors
- **Protection from Shear Stress & Mechanical Forces** during downstream process unit operations


**Exbumin - recombinant human serum albumin**

Exbumin is completely animal- and blood-free. It was developed to improve stability for vaccines and cell & gene therapies.

- FDA and EMA approved excipient for injectables
- Scalable, Sustainable and Reliable
- Made in the USA

### Increasing Viral, Thermal, & Chemical Stability

A key aspect of virus/vector production is maintaining stability and function of the final product, including sustained potency and viral titer. The presence of rHSA increases thermal stability and shelf-life.

For example, using excipients, like Exbumin, to stabilize drug products is critical, especially for clinical applications in which small amounts of highly concentrated vectors are introduced into confined spaces.

Previous studies by Wiggen et al. showed stability benefits of rHSA in flavivirus vaccines for Dengue. Reconstituted DEN-2 PDK-53 vaccine was evaluated for stability using various excipients, including common sugars, poloxamers, and Exbumin rHSA.[3,4]

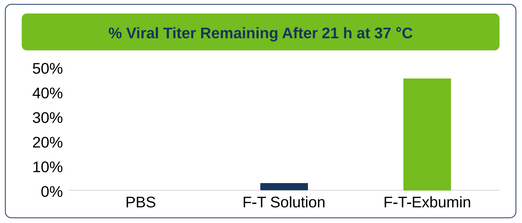



*Figure 2. Exbumin® rHSA, when combined with F127 copolymer and trehalose, provides significant virus survival, particularly when compared to PBS, which showed no surviving virus after 21 hours.[3,4,5]*

### Improving Recovery During Filtration

**In the figure below, we also demonstrate** recovery of functional virus (TU) post sterile filtration of the TFF concentrate. X-axis represents g/L of Exbumin added in the equilibration buffer at the start of TFF concentration. Percent recovery was calculated as total functional virus (TU) post sterile filtration over total TU post TFF recovery.

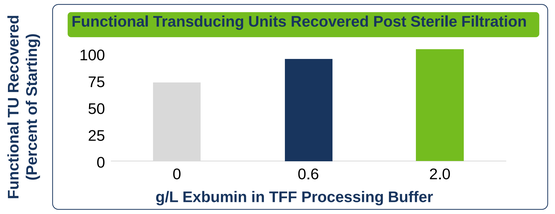



*Figure 3. These results suggest that the inclusion of recombinant albumin in the equilibration buffer added to a tangential flow cassette further increased downstream recovery of the lentivirus, increasing recovery approximately 15% compared to no albumin, and further recovering 100% of the lentivirus functional vectors during sterile filtration.[6]*

### Prevention of Aggregation Resulting in Higher Viral Activity and Titers

Hosokawa et al. demonstrated that aggregation of recombinant virus is responsible for the observed loss in adenovirus activity following filtration. The addition of 1% albumin prevented viral aggregation and allowed the purified virus to retain its activity after filter sterilization. Furthermore, viral activity was retained within the 1% albumin solution for at least 1 week at 37°C and for 2 weeks at 4°C, whereas viral activity with the albumin-free solution was quickly lost. These results indicate the potential usefulness of recombinant albumin in the preparation of recombinant adenovirus.[7]

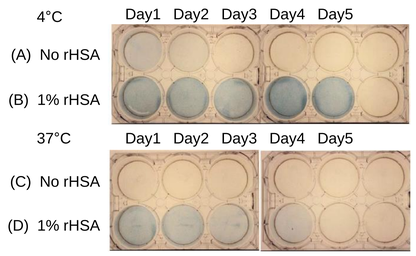



*Fig. 4. Effect of rHSA on the stability of AxCALacZ shows the LacZ activity of viral solution without rHSA at 4°C, (B) shows activity with 1% rHSA at 4°C, (C) shows the LacZ activity of viral solution without rHSA at 37°C, (D) shows activity with 1% rHSA at 37°C. Viral activity was retained within the viral solution containing 1% rHSA, but was quickly lost in rHSA free solution.[8]*

### Virus Stabilization during Fill & Finish, Cryopreservation, and Delivery

The low stability of secreted viruses, including gammaretroviral and lentiviral vectors, makes high quality clinical preparations difficult to achieve. Carmo et al shows that it is possible to increase the infectivity stability by adding rHSA to storage buffer, both at 37°C and 4°C. The half-life of LVs range from 5-8 hours in the cell culture supernatant, due to their labile nature, affecting not only the titers but also the quality and efficacy of vector for preparations. In the first stabilization test, BSA, HSA, and rHSA were tested in buffers alongside Tris 10, Tween, and MgCl2. Because recombinant human albumin is considered a safer component than animal albumin, the remainder of the studies were performed using rHSA. Albumin was shown to associate tightly with cell surfaces and vector membranes, creating stabilized complexes. From there, formulation studies showed that the combined use of lipoproteins and recombinant human serum albumin considerably improves stability of LVs at 4° and 37°C.[9] This association may provide protection of their structure and prevent conformational changes.

### Reducing Non-Specific Adsorption

Bandiera et al. reported on alternative strategies for the purification of lentiviral vectors using albumin membrane coating technologies, including depth-filtration, ultrafiltration/diafiltration, and anion-exchange chromatography. They also demonstrate the use of HSA in final formulations before freezing of final drug substance. Vivaflow cassettes were previously saturated with 20m1 (4g/L) of human serum albumin (HSA) (Sigma) for approximately 3 min, at a flow rate of 7 ml min-1, in order to prevent unspecific adsorption of the LVs to the membrane. This strategy led to infectious vector recoveries if up to 80%, about 10% higher than values currently reported in the literature.[9]

A cryoprotecting formulation of 0.5 M of sucrose (Merck) and 0.6 mg/m1 of HSA was added to the viral samples before freezing to reduce loss of infectious particles. In order to analyze the effect of the addition of these formulations, several aliquots (with an already known infectious titer) were frozen at -85°C with and without this cryoprotecting formulation. After 24hr samples were thawed on ice and their infectious titer was quantified by the method described in the section "Analysis of viral titers." Analysis of the LVs infectious titer after freeze-thawing without HSA revealed a drop of 84±3%, whereas samples stored in a formulation containing sucrose and HSA had a loss of only 26±2% of infectious particles after freeze-thawing.[10]

## CONCLUSIONS

- **Downstream processing holds many challenges for manufacturers, some of which include: preparing stable viral vectors, preventing degradation during manufacturing, handling and storage, and maintaining their long-term stability and efficacy.**
- **Albumin, among its many benefits, has been shown to significantly reduce vector and virus loss during downstream process unit operations.**
- **Recombinant Albumin (rHSA) is shown to consistently perform comparably, if not better, than native derived HSA.**
- **Exbumin® provides significant regulatory and quality benefits over other excipients, surfactants, and animal-derived reagents.**

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