Retain and Protect Final Cell Product with Optibumin®, a Recombinant Human Serum Albumin (rHSA)

Application Note

Mark Stathos, PhD, Product Applications Scientist, InVitria, Inc.

Andrew Hamann, PhD, Product Applications Scientist, InVitria, Inc.

Key Points

- Optibumin®, InVitria's high-purity recombinant human albumin, improves viable T cell retention during downstream washing.
- Optibumin performs comparably to clinical HSA in this application, enabling a like-kind replacement.
- Optibumin is animal-origin-free, offering safety and regulatory advantages.

Introduction

Critical Role of Albumin in Cell Washing for Cell Therapy

Cell washing occurs at several points during a typical cell therapy manufacturing process, including cell isolation, media changes during expansion and downstream processing prior to cryopreservation. Although the specifics of each operation vary slightly, they all share the common goal of removing unwanted components while maintaining a high number of viable cells. To achieve this, specialized buffers are used, and critically, albumin is frequently added to enhance their performance.

Albumin derived from human serum (HSA) is the most common source of albumin for this application; however, HSA presents several challenges related to safety, uniformity,

supply chain reliability, and regulatory compliance (Duarte, 2023; MacLennan & Barbara, 2006; Lu, 2024). Regulatory agencies are increasingly encouraging pharmaceutical companies to adopt animal-origin-free (AOF) materials for therapeutic production (U.S. FDA, 2024).

To address these issues, InVitria has developed Optibumin, a recombinant HSA (rHSA) solution engineered to mimic clinical HSA. In addition to being animal-origin-free, InVitria offers Optibumin 25 in bags that are compatible with closed-system cell manufacturing workflows, as well as in septum and screw cap bottles for flexible applications. Optibumin is manufactured in cGMP conditions in an ISO 9001:2015-certified facility, meeting stringent quality standards for clinical-grade materials. InVitria's scalable expression system and vertically integrated supply chain ensures a reliable source of high-quality rHSA, which is essential for uninterrupted cell therapy production. Here, we demonstrate the performance of Optibumin in a downstream T cell wash application.

Results and Discussion

Optibumin Improves T Cell Retention During Downstream Washing

Primary human T cells from two healthy donors were thawed and activated for three days before being plated into 96-well plates, counted well by well using high-content screening, and washed three times in 1% or 5% Optibumin or clinical serum-derived HSA in PBS or in PBS alone. Washes were conducted by repeated centrifugation, aspiration, and resuspension via pipette mixing. Though this method is somewhat more stressful to cells than washing with specialized devices, it remains an industrially relevant washing procedure (Li, 2021; Ibenena, 2022).

After washing, cells were held in the wash buffers for two hours to simulate industrially relevant holding times. Cells were then counted post-wash using the same high content screening method, and the percentage of viable cells retained was calculated.

Results for washes with 1% albumin and PBS alone are shown in **Figure 1**; results for 5% albumin were similar and were omitted for brevity.

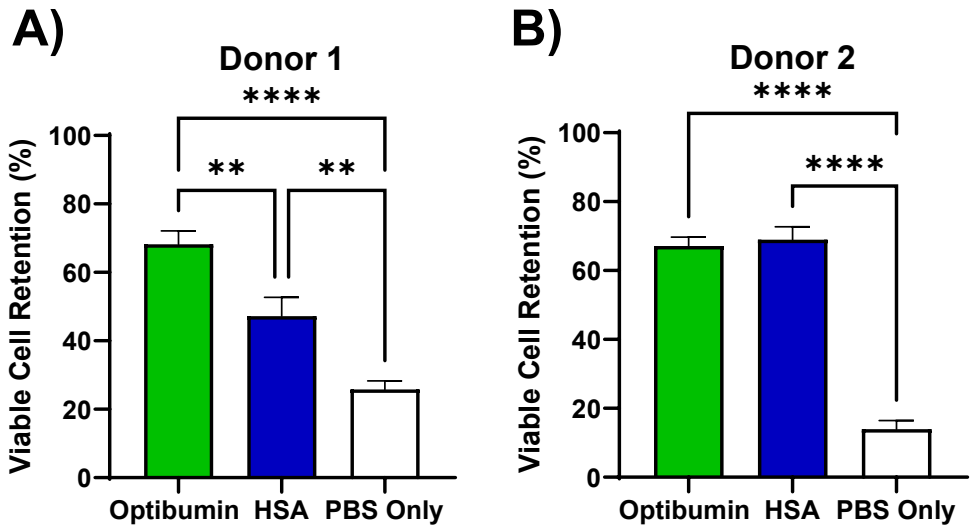


Figure 1: Viable cell retention post wash relative to initial viable cells present pre-wash for A) Donor 1 and B) Donor 2 when washed with 1% Optibumin or HSA or with PBS only (One way ANOVA with Tukey post hoc test ** $p < .01$, **** $p < .0001$)

These results demonstrate that both Optibumin and HSA significantly improve viable cell retention. Optibumin increased retention from 25% to 65% for Donor 1 **(Figure 1A)** and 15% to 65% for Donor 2 **(Figure 1B)** during downstream wash compared to PBS alone. Furthermore, Optibumin performed significantly better than HSA in the Donor 1 group and comparably in the Donor 2 group.

Conclusion

This study demonstrates that Optibumin, a recombinant human serum albumin (rHSA), reduces the loss of critical cell therapy final product during processing. As demonstrated by the retention data, the presence of albumin substantially improves viable cell retention while maintaining comparable viability. Taken together, Optibumin performs as well if not better than HSA in downstream cell washing applications.

As an animal-origin-free solution, Optibumin meets stringent regulatory requirements while addressing safety and supply chain challenges commonly associated with serum-derived albumin. Its compatibility with closed-system workflows and scalable production processes further enhances its utility for modern cell therapy manufacturing. Collectively, these attributes position Optibumin as a robust and reliable alternative to clinical-grade HSA, offering consistent performance for CAR-T and other adoptive cell therapy applications.

Materials and Methods

Cell Culture and Activation:

Primary T cells were isolated from leukopaks derived from 2 healthy, non-smoker donors aged between 18 and 65 years with a BMI <30. Isolation was performed using a negative selection kit following the manufacturer's protocol. The isolated cells were cryopreserved in liquid nitrogen using a formulation containing 10% DMSO.

Before the experiment, cells were thawed and adjusted to a density of 1.0×10^6 cells/mL in serum-free T cell media supplemented with 200 IU/mL of IL-2. Activation was initiated by adding a CD3 and CD28 antibody cocktail to the cells and incubating them for three days.

Cell Washing:

After three days of activation, 10 mL aliquots of the specified wash buffer conditions were prepared. The primary human T cells were counted using an automated cell counter, and aliquots of 2.5×10^5 live cells were transferred into 96-well V-bottom plates. Cells were pelleted by centrifugation at 500 × g for 5 minutes, after which the supernatants were carefully aspirated. The cell pellets were then resuspended in 200 µL of the corresponding wash buffer by vigorous pipetting.

This washing procedure was repeated two additional times, resulting in a total of three washes. During the final wash, the cells were held in the wash buffer for 2 hours to

simulate a typical hold time encountered in cell therapy manufacturing process (Pattasseril, 2013).

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For additional product or technical information, please contact us at

2718 Industrial Drive
Junction City, KS 66441

Phone: 1.800.916.8311
Email: Info@InVitria.com

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