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Application Note

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Virus-Like Particles Purification and Clarification Using Ksep® 50 Counterflow Technology

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Abstract

Virus-like particles (VLPs) are gaining traction as innovative preventive therapeutics in immunization. Their potential as advanced drug delivery systems is also the focus of intense research. As a result, there is a rising demand for robust and reliable downstream processing methods for VLPs. Sartorius´ Ksep® counterflow centrifugation platforms are characterized by their gentle and efficient separation of VLPs from the cell culture broth, achieving high yields and purity in VLP recovery. The highly automated Ksep® technology reduces handling errors, and its closed system, featuring sterile single-use consumables, sealed bags and tubes, prevents external contamination.

This application note details the downstream processing of VLPs using the Ksep® 50 counterflow centrifuge, suitable for VLP-containing cell culture volumes from 0.1 to ~20 L. It enables downstream processing of smaller volumes in research applications or as a scale-down model before transitioning to large-scale production using the Ksep® 400.

For further information, visit
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Introduction

Virus-like particles (VLPs) are nanoparticles produced by cells and can be categorized into two types: enveloped and non-enveloped. While they originate from viruses, VLPs lack a viral genome and are non-infectious, as they do not have the ability to replicate. In recent years, VLPs have gained significant importance in the field of vaccination. Both types of VLPs are also employed in gene therapy, drug delivery, and diagnostic assays due to their ability to elicit strong immune responses and their customizable surface properties. The adaptability and safety profile of VLPs make them a promising platform for various therapeutic and preventive applications. In addition, the structural similarity between enveloped VLPs and exosomes, which are extracellular vesicles involved in cell communication, has sparked interest in using enveloped VLPs as a model for exosome research.

As the significance of VLPs in clinical applications grows, the biopharmaceutical industry urgently requires the development of efficient downstream processing methods for their production. This is essential to achieve high-throughput manufacturing that consistently delivers optimal quantities of high-quality and high-purity VLPs. However, the purification of enveloped VLPs is particularly challenging due to their structural complexity and fragility, necessitating more sophisticated and gentle purification techniques.

The Ksep® 50 is a single-use (SU) counterflow centrifuge ideal for small-scale production, handling bioreactor volumes starting at 0.1 L. It allows seamless scaling up to the Ksep® 400 for volumes up to 200 L. Ksep® centrifuges efficiently separate VLPs from cells using a fluidized bed and counterflow, ensuring low shear forces and high recovery rates. This automated SU technology supports continuous VLP separation in cGMP-compliant processes from from early development to commercial scale.

Functional Principle of Ksep® Technology

To separate the VLP-containing supernatant from cells, the Ksep® 50 is operated in harvest clarification mode. The bioreactor broth is processed in cycles consisting of the following steps:

1. Concentration step: Bioreactor broth is pumped through conical chambers in the spinning centrifuge rotor. The generated *g*-force pushes the cells outward to the rotor's edge. Simultaneously, the incoming flow of cells counteracts this force, creating a fluidized cell bed and allowing the VLP-containing medium to pass through the chamber and be collected separately from the cells. The balance of forces minimizes shear stress, preventing cell damage and reducing the release of cellular impurities into the medium (Figure 1A).

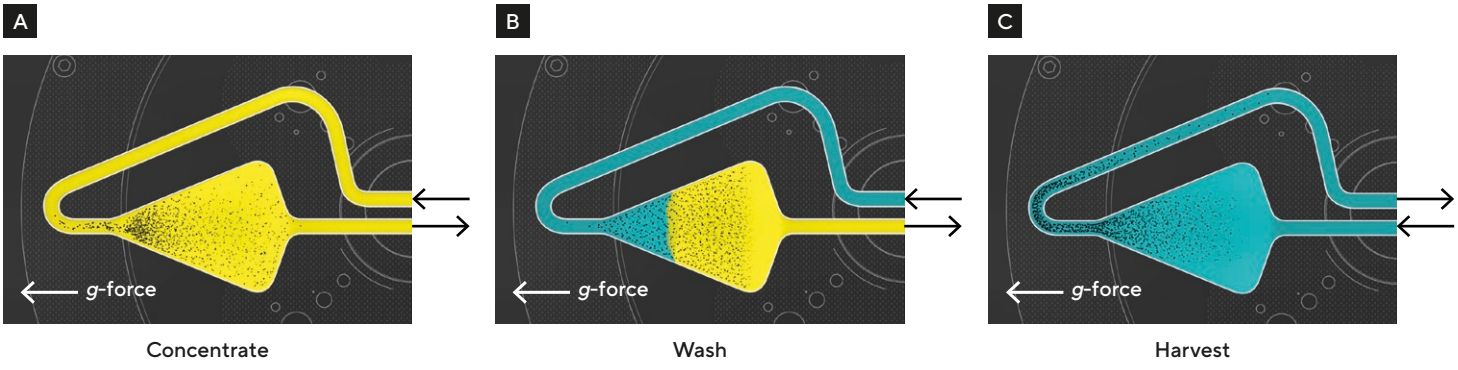
2. Washing step: Once a sufficient number of cells have accumulated, wash buffer replaces the remaining VLP-containing medium in the chambers. The precise timing of the wash volume minimizes product dilution (Figure 1B).

3. Cell discharge step: The flow direction is reversed to remove the cells from the chamber, and the next cycle begins (Figure 1C).

The Ksep® 50 can process cell broth with input concentrations of approximately 2×10^8 cells/mL.

This document describes the downstream processing of enveloped VLPs, starting with harvesting and clarification of VLPs using the Ksep® 50 SU counterflow centrifuge, followed by further clarification of the harvested VLP fraction using Sartopure® PP3 and Sartopore® 2 filters.

Figure 1: *Functional Principle of Ksep® Technology*



Note. **(A)** Cell broth (yellow) is pumped through a chamber, with cells being concentrated as a fluidized bed while separating VLP-containing medium. **(B)** Wash buffer (blue) is pumped through the fluidized bed to wash out remaining VLP-containing medium. **(C)** Flow direction is reversed for cell removal before the next concentration cycle starts.

Materials and Methods

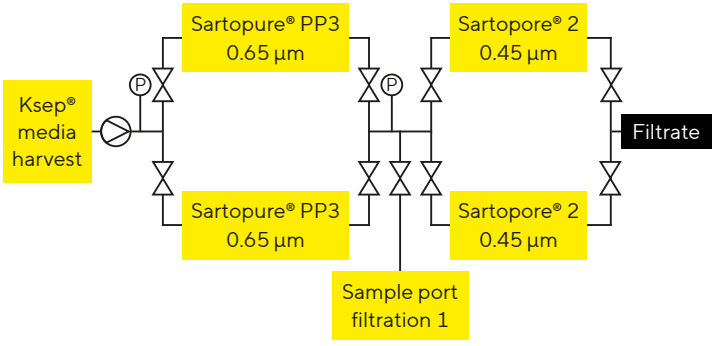
VLPs Production

Enveloped Mos1-Gag-GFP VLPs were stably produced using HEK293-F cells cultured in 4Cell® SmartCHO Production Medium (Sartorius) and a 10 L Univessel® Glass Bioreactor (Sartorius). Bioreactor process parameters were controlled by a Biostat® control unit (Sartorius) at 37 °C, 40% dissolved oxygen, pH ≤ 7.25, and 202 rpm. Prior to harvest, at 144 h post-inoculation, the culture was treated with 10 U/mL DENARASE® nuclease (c-LEcta) and 2 mM $MgCl_2$ for 1 hour under the set process parameters.

Ksep® Harvest and Clarification

For downstream processing, 2 L of cell broth was processed using the Ksep® 50 with PBS as wash buffer. Post-centrifugation clarification involved two stages of particle filtration: the first filter stage utilized Sartopure® PP3 Midicaps 0.65 µm, and the second stage employed Sartopore® Midicaps 0.45 µm (Figure 2). After filtration, the filter train was flushed with 900 mL of PBS.

Figure 2: *Schematic of the Post-Ksep® Filtration Setup*



The parameters for the recovery of VLPs with the Ksep® 50 were selected based on our previous process experience with HEK293 cells. One chamber of the Ksep® 50 centrifuge was used at 2,000 × *g* and a flow rate of 73 mL/min for concentrating and washing. The filling of the chamber with a fluidized cell bed was initially monitored empirically to determine chamber filling, and reached approximately 95% at a volume of 0.42 L. The concentration step was then completed manually, and in subsequent cycles, the volume of 0.42 L was processed automatically. Each cycle was followed by a wash with PBS buffer at the equivalent of 2.6 chamber volumes. Table 1 lists all process parameters utilized in the harvest clarification phase of the Ksep® 50.

Table 1: *Process Parameters Used in the Ksep® 50 Harvest Clarification Process Phase*

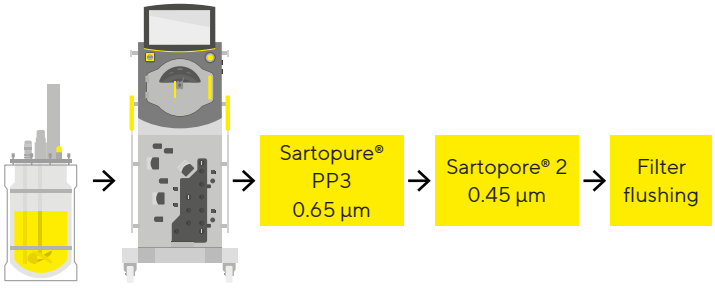
Ksep® 50 Parameter	Unit	Setting
Chamber selection (0 = A, 1 = A & B)	—	0
Normal speed centrifuge	× *g*	2,000
Starting flow rate	mL/min	50
Flow rate ramp time	s	30
Establish bed duration timer	min	0.1
Normal rate chamber pumps	mL/min	73
Cycle volume per chamber*	L	(1) → 0.42
Wash 1 volume	n/a	2.6
First wash chamber pump	mL/min	73
Harvest and mix speed centrifuge	× *g*	2,000
Harvest flow rate chamber pump	mL/min	80
Initial dump volume	mL	15
Harvest volume	mL	80

*Cycle volume per chamber describes the volumetric amount processed from the bioreactor inlet during the concentration step per cycle. An initial value of 1 L was entered, which was intentionally much larger than the expected cycle volume. During the first cycle, the bed formation and chamber filling with the fluidized cell bed was observed through the inspection window. At ~95% chamber filling height, the concentration step was completed via operator input. The cell broth volume that had been processed up to that point (0.42 L) was thereby automatically entered as new value and used in all further consecutive cycles.

Results and Discussion

The overall process setup is illustrated in Figure 3.

Figure 3: *Overview of VLP Processing Using the Ksep® 50 Counterflow Centrifuge and 2-Step Filtration*



Analytics

VLP concentrations were determined using fluorescence-based nanoparticle tracking analysis with a NanoSight NS300 (Malvern Panalytics) equipped with a 488 nm laser and a 500 nm long-pass filter. Turbidity was measured with a TL2350 turbidity meter (Hach). Cell density and viability were quantified using the Cedex® HiRes Analyzer (Roche Diagnostics).

Sampling

The efficacy of VLP purification with the Ksep® 50 was assessed by analyzing samples collected at feed, waste, and harvest stages to determine VLP concentration and yield. Cell concentration and viability were only evaluated for feed and harvest stages, while turbidity was measured in all samples. The effectiveness of the downstream filtration process was evaluated by collecting samples from the filtrate after the initial filtration step (0.65 µm | Filtration 1), the filtrate after the second filtration step (0.45 µm | Filtration 2), and the final filtrate following the post-flush. Relevant samples were analyzed for turbidity and VLP concentration.

Throughput

Ksep® 50 achieved a throughput of 3.39 L/h with one chamber. Using both chambers enables a throughput of 6.78 L/h with the given process parameters.

Processed Volumes and Dilution

Table 2 outlines the volumes processed during VLP harvesting and clarification, while Table 3 provides the volumetric dilution factors calculated to assess dilution occurring during the centrifugation and filtration processes. VLP harvesting with Ksep® 50 resulted in a slight dilution of only 1.13-fold. The volume of the filter train post-flush was approximately equivalent to the end-to-end hold-up volume, indicating that no additional product dilution was introduced by the filtration step, with dilution factors of ~ 1 after the post-flush. Over the entire process, the dilution factor was only 1.15. Notably, the filtration did not cause any further dilution due to the low post-flush volume.

Table 2: *Processed Volumes During the Clarification of VLP-Containing Cell Broth Using the Ksep® 50 Counterflow Centrifuge*

Process Steps	Volume [mL]
Feed	1,978
Waste	1,222
Harvest	2,233
Filtration 2	1,379
Post-flush	2,280

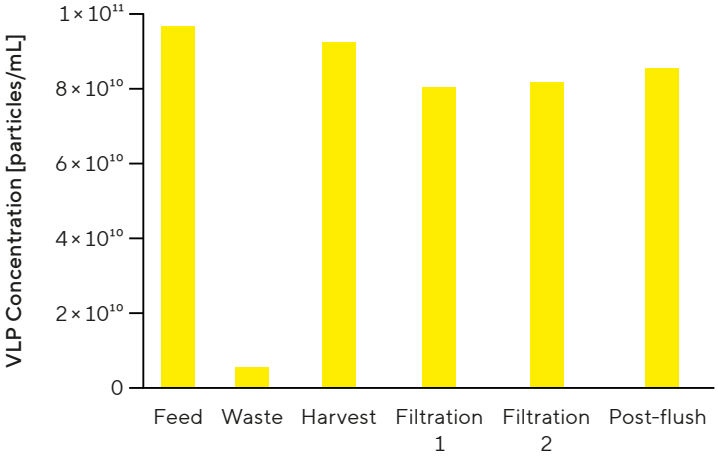
Table 3: *Volumetric Dilution Factors for Individual Process Steps and the Total Process*

Process Steps	Dilution Factor
Feed → harvest	1.13
Feed → post-flush	1.15
Harvest → post-flush	1.02

VLP Concentration and Total Yield

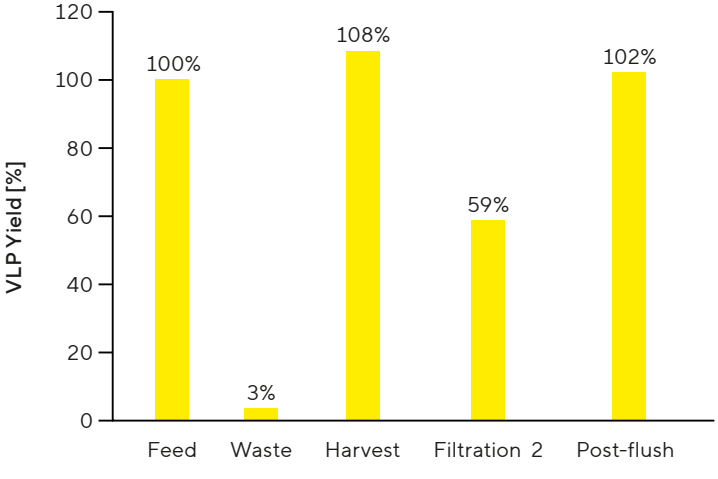
Throughout the entire process, the VLP concentration decreased only slightly, demonstrating the efficiency of the system. In contrast, the Ksep® 50 waste exhibited low VLP concentrations, underscoring the exceptional separation capabilities of the Ksep® 50, as shown in Figure 4.

Figure 4: *VLP Concentrations at Different Process Steps*



VLP yields were calculated based on measured VLP concentrations and processed volumes. After harvesting, the total number of VLPs increased slightly, likely due to variations in measurement accuracy of the analytical method. The Ksep® 50 waste sample showed a low VLP yield of 3%. The overall VLP yield of ~ 100% indicates no significant loss compared to the feed (Figure 5). These results suggest that the Ksep® parametrization, with a wash volume of 2.6 chamber volumes, was highly effective in replacing the supernatant in the cell bed while achieving minimal product dilution.

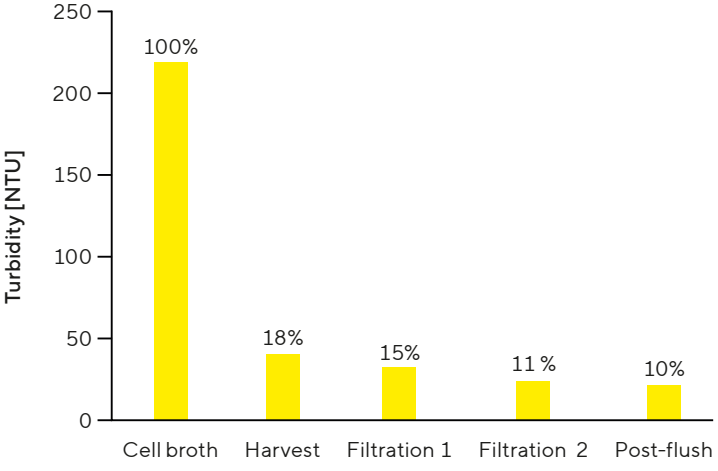
Figure 5: *VLP Yields at Different Process Steps*



Turbidity

The high performance of the Ksep® 50 was confirmed by turbidity measurements (nephelometric turbidity units; NTU), as seen in Figure 6.

Figure 6: *Turbidity Decreases With Downstream Processing*



With an initial turbidity of 219 NTU in the cell broth, the reduction to 40 NTU in the harvest demonstrates highly efficient clarification by the Ksep® 50. This is emphasized by the fact that the turbidity was only reduced by a further 8 NTU by the 0.65 µm filter (Filtration 1). The additional 0.45 µm filter (Filtration 2) resulted in a final value of 22 NTU in the post-flush, representing an overall turbidity reduction of 90%.

Cell Removal

The low turbidity in the harvest indicated effective cell removal, which was confirmed by measuring viable cells in the feed and the harvest. Ksep® 50 processing led to a substantial three-log reduction in the concentration of viable cells, as shown in Table 4.

Table 4: *Cedex Cell Counter Results*

	Viability [%]	Viable Cell Concentration [cells/mL]	Viable Cell Count
Feed	89.02	3.73×10^6	7.38×10^9
Harvest	11.54	2.67×10^3	5.95×10^6

Having confirmed the effective removal of cells through a substantial reduction in viable cell concentration, the next focus was evaluating the filtration process, particularly the pressure dynamics involved.

Filtration Pressure
The pressure upstream of the filters was measured to estimate the extent of blockage during harvest filtration. After venting of the pre-wetted filters, the filtration was conducted at a flow rate of 24 L/h.

A back pressure of 0.35 bar occurred upstream of the first filter stage, where the cumulative pressure of both filter stages is combined. Further reduction (after the initial adjustment period) measured upstream of the first filter (Sartopure® PP3 0.65 µm) throughout the 2.23 L filtration highlights Ksep® processings exceptional separation capabilities. The second filter stage (Sartopore® 2 0.45 µm) maintained significantly lower back pressures, with a maximum of 0.08 bar throughout the filtration process. During the post-flush, the back pressure in the first filter stage slightly decreased to 0.25 bar. Both filter types are typically operated at pressures of up to 1.5 bar.

Conclusion

The increasing use of VLPs in vaccinology, coupled with extensive clinical research into their potential as drug delivery systems for advanced therapies, underscores the need for a streamlined pipeline for their efficient purification and clarification.

The Ksep® 50 is a superior SU counterflow centrifuge, capable of effectively separating enveloped, extracellular VLPs from producer cell cultures under sterile conditions.

Analytics following Ksep® 50 processing and subsequent filtration of the harvest demonstrated VLP yields close to 100% of the initial feed concentration, with minimal overall dilution at approximately 1.1. The Ksep® 50 significantly reduced turbidity, demonstrating its outstanding separation capabilities even prior to filtration. Its capability to process small to medium quantities streamlines production processes, allowing for the identification of critical process parameters on a smaller scale before seamlessly scaling up to the Ksep® 400 platform for larger production volumes. With sterile SU consumables and flexible options for both automated and manual operation, it marks a significant advancement in cGMP-compliant downstream processing of VLPs, ensuring high recovery rates and excellent purity in small to medium-scale applications.

The similarity of the enveloped VLPs used in this trial to enveloped viruses and exosomes means they also serve as a model for these particles. As a result, the excellent results achieved in this trial are potentially also applicable to these modalities.

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