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Application Note

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Enhancing Downstream T Cell Processing With Ksep® Single-Use Counterflow Centrifugation

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Abstract

T cells are increasingly employed in advanced therapies and are the focus of extensive research into new clinical applications. To meet the growing demand, the bioprocessing industry must implement solutions that support their reliable and robust downstream processing.

Ksep® systems are automated, single-use solutions specifically designed for closed-cell processing. They feature scalable, continuous counterflow processing for aseptic cell concentration, washing, and harvesting, and their low shear force and effective separation, maximize recovery rates while ensuring cell viability.

This application note demonstrates the suitability of the Ksep® 50 counterflow centrifuge for the efficient separation of T cells from cell culture media. The purified cells can be used for seed train-like upscaling, further downstream processing, or as a high cell concentrate when eluted in a small volume, which is essential for electroporation to produce genetically modified T cells.

For more information about Ksep®, visit
www.sartorius.com/ksep

Introduction

T cell therapies have proven to be a significant advance in cancer treatment, particularly chimeric antigen receptor T (CAR-T) cell and T cell receptor (TCR) therapies. Ongoing clinical research is also exploring the potential of T cell-based therapies for other indications, such as auto-immune diseases, infectious diseases, and transplantation.

As the demand for T cells in medical applications continues to rise, the biopharmaceutical industry needs to establish robust production processes that ensure high-quality, aseptic T cell manufacturing. Achieving optimal yields and high throughput is crucial for maintaining cost efficiency. Moreover, the scalability of downstream processing platforms is essential for optimizing critical process parameters at a small scale before transitioning to commercial manufacturing.

Ksep® single-use counterflow centrifugation systems provide an effective and efficient solution for closed-cell processing, including concentration, washing, and harvesting (Figure 1). Their dual functionality supports the harvest of cells as a product and the removal of cells for media harvest or clarification. The automated single-use design allows the Ksep® systems to process cells within a closed unit using sterile disposables, minimizing the risk of contamination and handling errors.

Functional Principles of Ksep® Technology

To harvest cells from bioreactor broth, the Ksep®50 is operated in concentration, wash, harvest (CWH) mode. The bioreactor is processed in cycles consisting of the following steps:

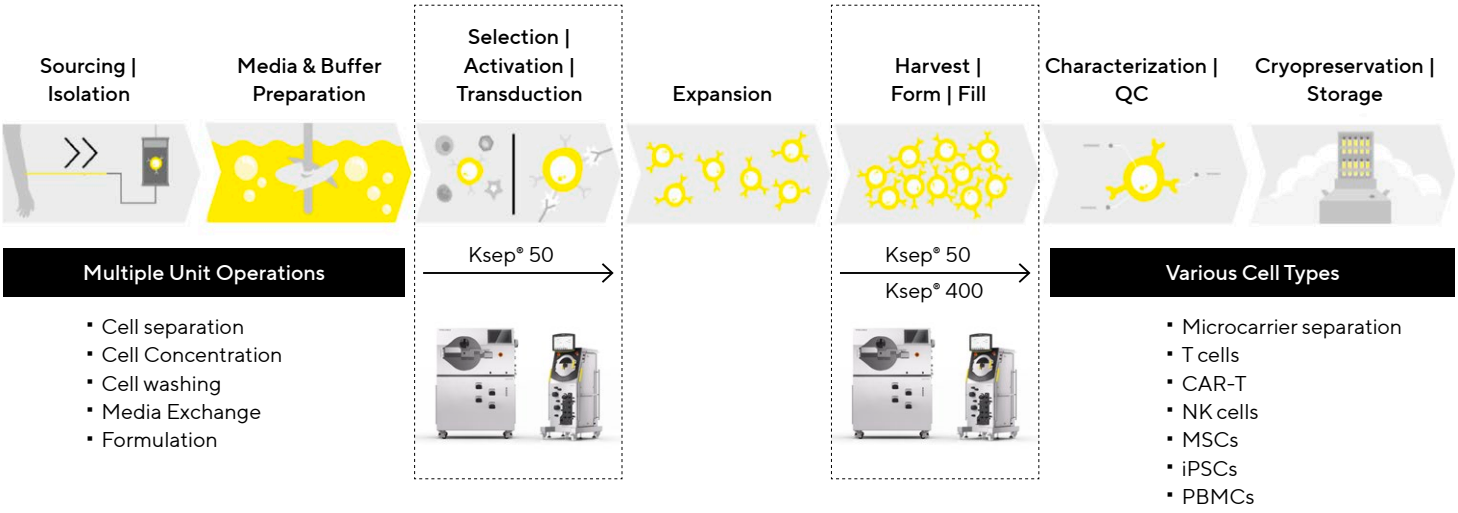
1. **Concentration:** Bioreactor broth is pumped through conical chambers in the spinning centrifuge rotor. The g-force pushes the cells outward to the rotor's edge, while the flow of cells counteracts this force, creating a fluidized cell bed. This balance ensures minimal shear force, preventing cell damage while effectively removing the majority of cell debris and dead cells (Figure 2A).

2. **Washing:** Once a sufficient number of cells have accumulated, wash buffer replaces the bioreactor broth (Figure 2B) further aiding the removal of debris.

3. **Harvest:** The flow direction is reversed to collect the cells from the chamber, and the next cycle begins (Figure 2C).

The Ksep® 50 can process cell broth with input concentrations of approximately 2×10^8 cells/mL. The Ksep® system effectively retains particles larger than 5 µm, allowing for the efficient separation of cells from smaller particulates.

Figure 1: *The Ksep® Platform Can be Used for Multiple Unit Operations Throughout the Manufacturing Of Various Cell Therapies, Including T Cells*



Materials and Methods

This process allows for the precise separation of specific components while minimizing shear forces, resulting in high recovery rates of viable cells. The scalable Ksep® portfolio includes counterflow centrifuges suitable for processing various cell culture volumes, ranging from early-stage research and process development and to large-scale cGMP production.

This application note outlines the suitability of the Ksep® 50 SU counterflow centrifuge in the downstream processing of T cells for advanced cell therapies. Two runs were performed with different objectives:

Run 1:
Optimization of T Cell Concentration, Wash, and Harvest
The first process run focused on the concentration, washing, and harvesting of high-quality T cells. These cells are intended for further purification in downstream processing or for use in cell expansion in a seed train.

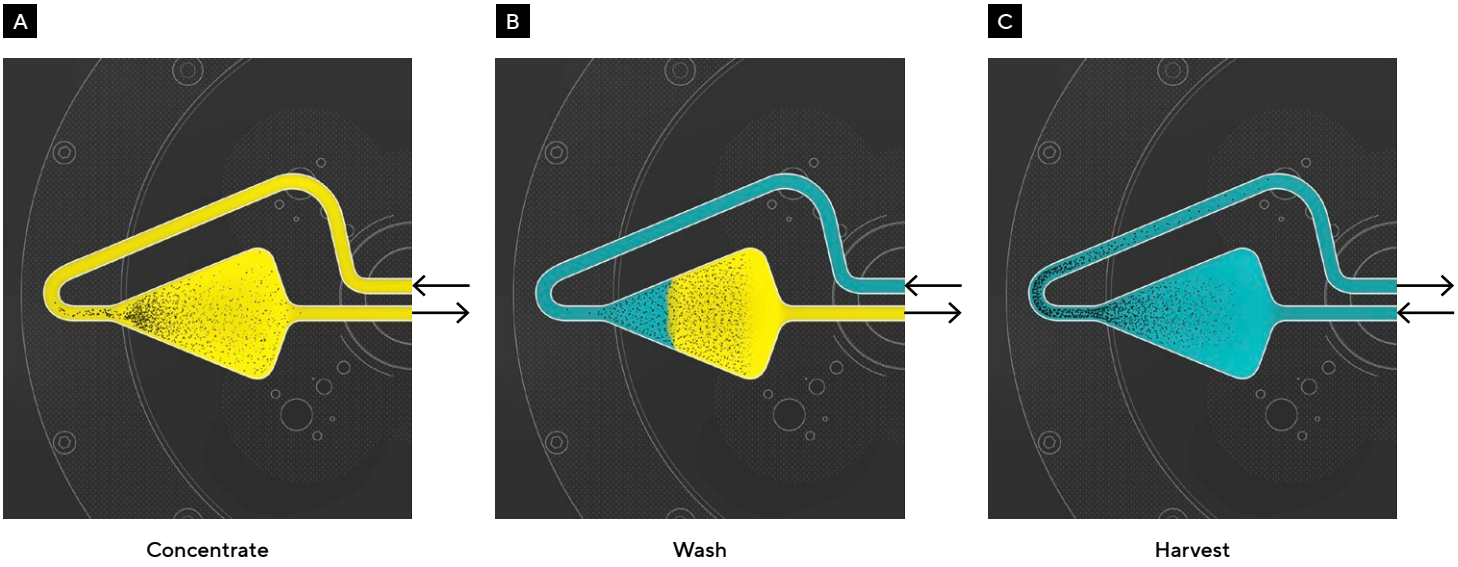
Run 2:
Ultra-High Cell Density Concentration, Wash, and Harvest
The second process run demonstrated the suitability of the Ksep® 50 for concentrating T cells to a very high cell concentration. This cell density is essential for the subsequent engineering of T cells, such as through electroporation, to produce genetically modified cells.

T Cell Cultivation and Feed Preparation

Human peripheral blood mononuclear cells (PMBC, T cells) were cultured in a Biostat® RM TX Bioreactor (Sartorius) utilizing Flexsafe® RM 2 L perfusion bags (Sartorius) with LymphoONE™ T Cell Expansion Xeno-Free Medium (Takara Bio) with 5% human serum and 200 U/mL IL-2 added to the media. Throughout the cultivation period, the temperature was maintained between 35.5 and 37.5 °C, the pH values ranged from 7.0 to 7.5, and the dissolved oxygen levels were kept at > 20% air saturation. The average size of the T cells was approximately 8 µm.

The cell broth was diluted to match specific test case parameters required for this trial. For the first run, 0.9 L of cell broth was diluted with LymphoONE™ media, supplemented with 5% human serum, to a concentration of 10×10^6 cells/mL. About 1 L of the diluted cell culture (feed), containing ~ 10×10^9 cells, was used for subsequent downstream processing with the Ksep® 50 SU. For the second run, 0.95 L of cell broth was diluted as described above to a concentration of 24×10^6 cells/mL prior processing with the Ksep® 50.

Figure 2: *Functional Principle of Ksep® Technology*



Note. **(A)** Cell broth is pumped through a chamber with cells being concentrated as a fluidized bed. **(B)** The wash buffer is pumped through the fluidized bed to wash the cell bed. **(C)** Flow direction is reversed for cell harvest before the next concentration cycle starts.

Ksep® 50 Parameters

During Run 1 the Ksep® 50 was operated at 2,000 × *g*, utilizing one of the two chambers (Ch) at a flow rate of 60 mL/min. During the initial cycle, the filling of the chamber with the fluidized cell bed was visually monitored to determine the processable volume per cycle. After processing 0.5 L, the chambers were filled to 90% with cells. The concentration step was then completed before moving on to the wash step. In the next cycle, the volume of 0.5 L/Ch was processed automatically with a cycle time of 13.5 minutes.

Run 2 was conducted similarly, using a chamber filling volume of 364 mL, a flow rate of 40 mL/min throughout the concentration and wash steps, and a harvest volume of 25 mL. The lower volume needed for filling the chamber was due to the higher cell concentration of the feed used for Run 2. This setup resulted in a cycle time of 9.5 minutes.

The most important parameters for both runs are summarized in Table 1. These parameters can serve as a starting point for similar experimental setups. Further optimization regarding variable flow kit connection lines and cell concentration may be necessary.

Table 1: *Overview of Relevant Ksep® 50 Parameter Settings for Run 1 and Run 2*

Ksep® 50 Parameter	Unit	Run 1	Run 2
Chamber selection (0 = A, 1 = A & B)	-	0	0
Normal speed centrifuge	× *g*	2,000	2,000
Normal rate chamber pumps	mL/min	60	40
Cycle volume per chamber	L	0.5	0.5
Wash 1 volume	ChV	5	3
First wash chamber pump	mL/min	60	40
Harvest and mix speed centrifuge	× *g*	2,000	2,000
Harvest flow rate chamber pump	mL/min	40	40
Initial dump volume	mL	5	20
Harvest volume	mL	100	25

Sampling and Analytics

The T cells collected during the first run were analyzed for cell viability and integrity. This included assessing the percentage of total, viable, and dead cells, aggregate formation, and cell size using a Cedex® HiRes Analyzer (Roche Diagnostics). Analysis was performed in duplicates using samples from the feed, the harvest (product), and the waste. Cell distributions were calculated by averaging both measurements. The maintenance of the T cell phenotype during downstream processing with the Ksep® 50 was evaluated through flow cytometric analysis of T cell-specific markers in feed and harvest samples, utilizing the IntelliCyt® iQue Screener PLUS (Sartorius). The effectiveness of the second run, aimed at ultra-concentration of cells in a minimal elution volume, was evaluated by analyzing duplicate samples from the feed, waste, and harvest. The total cell count and the proportion of viable cells were determined using the Cedex® HiRes Analyzer.

Results

Run 1: Optimization of T Cell Harvest

Throughput per Cycle

An average of approximately 5×10^9 cells were processed using one of the two chambers in a single cycle lasting 13.5 minutes, resulting in a T cell throughput of 3.7×10^8 cells/min. If both chambers are used, the Ksep® 50 throughput would double, reaching an estimated 7.3×10^8 cells/min. However, it's important to note that this throughput is specific to the T cell concentration in the feed and the selected Ksep® 50 parameter settings.

Process Time and Volumetric Throughput

In each 13.5-minute cycle, 514 mL of feed volume was processed using one of the two centrifuge chambers. If both chambers are used, the volumetric throughput would increase to 4.6 L/h. The single-use flow kit is validated for continuous operation for up to 8 hours. Therefore, with this approach, the Ksep® 50 would process approximately 36.6 L of cell broth with each single-use consumable set. Table summarizes the volumes, cell densities, and cell viabilities of the feed, waste, and harvest pool averages per cycle.

Table 2: *Results Summary for the Optimization of T Cell Harvest (Run 1) on Ksep® 50*

Sample	Volume [mL/cycle]	Cell Concentration [cells/mL]	Viability [%]	Viable Cell Yield [%]
Feed	514	9.6×10^6	95	-
Waste	795	0.6×10^6	62	-
Harvest	104	45×10^6	98	98

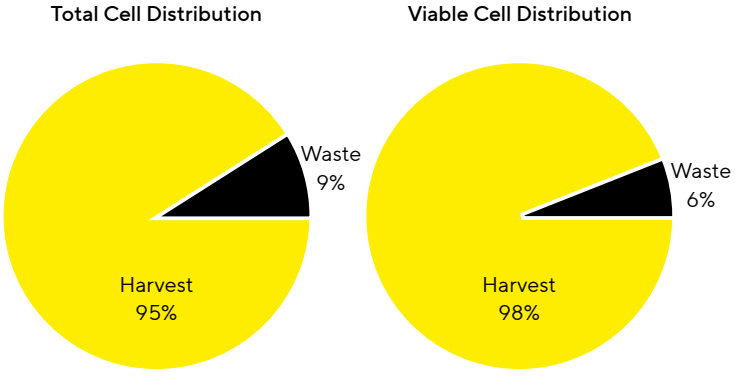
Note. Average volumes per cycle from two performed cycle. Cell concentration, viability, and yield were calculated from pooled samples.

Cell Sorting, Viability and Yield

Downstream processing with the Ksep® 50 achieved a high recovery of total T cells (both viable and dead cells) in the harvest, with a yield of 95% of the feed material. Only 9% of the total cells were found in the waste pool, demonstrating the excellent cell separation capability of the Ksep® 50. Similarly, viable cells were effectively enriched, with 98% of viable cells in the harvest and only 6% present in the waste (Figure 3). The sum of harvest and waste-bound cells showed only minor discrepancies in the mass balance, slightly exceeding the theoretical 100% yield. These variations are within the expected limits of the analytical methods.

Analyzing the percentage of viable cells—the primary product of interest—provides crucial insights into the system's suitability for the downstream processing of T cells. The gentle processing method, with minimal shear forces, showed remarkable effectiveness, achieving a viability rate of 98% in the harvested cells (Table 2). This marks an improvement over the 95% viability observed in the feed, underscoring the efficiency and potential for optimizing T cell processing with the Ksep® 50.

Figure 3: *Distribution of Total and Viable Cell Counts From Feed to Waste and Harvest During Run 1*



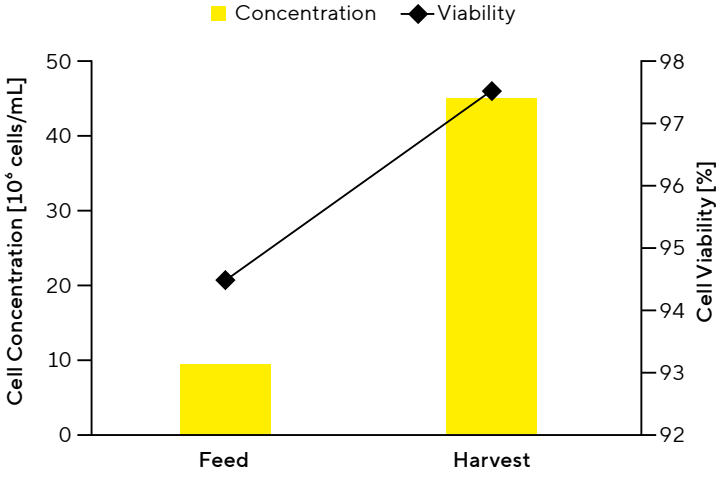
Note. The mass balance does not sum to exactly 100% due to technical limitations of the analytical assays.

The particle diameter is the key factor influencing the sedimentation rate. Smaller, collapsed, dead cells show significantly lower retention compared to viable cells. Since the effective linear counterflow velocity was equal to or slightly higher than the sedimentation velocity of the smaller dead cells, they were mostly not retained and washed out during concentration and washing. Of the dead cells present in the feed, only 43% were found in the harvest. The remaining 65% were cleared to the waste. This cell sorting effect was also evident in the significantly lower overall cell viability in the waste pool compared to the feed and harvest pools.

T Cell Concentration

During the two cycles, the feed volume of 1,028 mL was concentrated to a total harvest volume of 208 mL, achieving a volumetric concentration factor of 4.9. The viable cell concentration increased significantly from 9.6×10^6 cells/mL in the feed to 45×10^6 cells/mL in the harvest (Figure 4). This resulted in a cell concentration factor of 4.8, closely matching the volumetric concentration factor, underlining the exceptionally high viable cell yield. Additionally, the viability of the harvested cell population increased during the concentration process because dead cells were effectively directed to the waste.

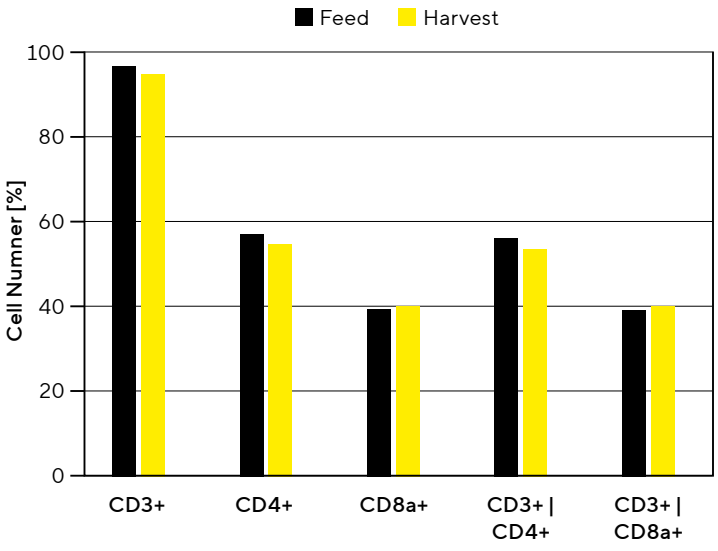
Figure 4: *Total Cell Concentration And Cell Viability of Feed and Harvest Pools From Optimization of T Cell Harvest (Run 1)*



Phenotype Maintenance

Figure 5 shows the results of the flow cytometric analysis of the expression of CD3, CD4, and CD8a T cell phenotype markers. Downstream processing with Ksep® 50 did not alter the T cell markers, as the distribution of CD3, CD4, and CD8a-positive T cells remained consistent during processing. Furthermore, phenotype markers had no effect on cell sorting, as evidenced by the high recovery rates.

Figure 5: *Distribution of CD3, CD4, and CD8a-Positive T Cells in Ksep® 50 Feed and Harvest Samples*



Run 2: Ultra-High Density Harvest

Throughput per Cycle

The throughput per cycle was calculated as described. With a feed concentration of 24×10^6 cells/mL and a cycle volume of 364 mL, 9×10^8 cells/min were processed per cycle using one chamber. Using both chambers would double the throughput to 1.8×10^9 cells/min.

Process Time and Volumetric Throughput

In the ultra-high concentration run, 364 mL of feed was processed per cycle using a single centrifuge chamber, with each cycle taking 9.5 minutes. If both chambers were used, the volumetric throughput would be 4.6 L/h, allowing for the processing of 36.8 L of cell broth with each single-use consumable set.

Total Cell Count and Viability

Table 3 summarizes the processed and received volumes for each cycle, along with the total cell count, viability analysis, and the calculated yield. The outstanding separation efficiency of the Ksep® 50 is demonstrated in the higher proportion of viable cells found in the harvest compared to the waste. Furthermore, approximately 91% of the viable cells from the feed stream were successfully retained in the harvest.

Discussion

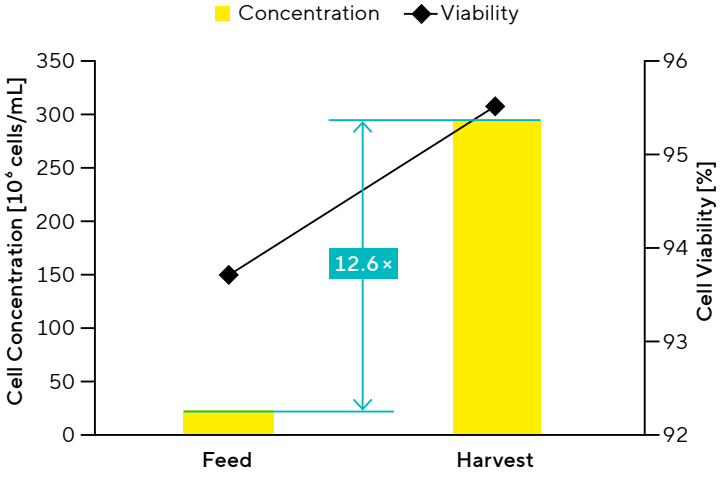
Table 3: *Results Summary for the Ultra-High Cell Density Run in Ksep® 50 (Run 2)*

Sample	Volume [mL/cycle]	Cell Concentration [cells/mL]	Viability [%]	Viable Cell Yield [%]
Feed	364	24×10^6	94	-
Waste	523	1.4×10^6	66	-
Harvest	26	295×10^6	96	91

Concentration

With a harvest volume of 26 mL per cycle, an approximately 14-fold concentration was achieved from a feed volume of 364 mL. Additionally, the concentration of viable cells per mL increased significantly by a factor of 12.6, rising from 24×10^6 cells/mL to 295×10^6 cells/mL (Figure 6).

Figure 6: *Total Cell Concentration and Cell Viability of Feed and Harvest Pools from Ultra-High Cell Density (Run 2)*



The growing importance of T cell therapies, especially CAR-T and TCR therapies, highlights the urgent need for efficient downstream processing methods for T cells in the biopharmaceutical industry.

In two Ksep® 50 runs, the T cell broth was concentrated significantly from initial densities of 10×10^6 cells/mL (first run) and 24×10^6 cells/mL (second run) to final densities of 45×10^6 cells/mL and 295×10^6 cells/mL, respectively. Cycle volumes were reduced from approximately 514 mL and 324 mL to final harvest volumes of 104 mL and 26 mL. These results demonstrate that the Ksep® 50 not only facilitates the washing, harvesting, and concentration of T cells as the initial step in seed train-like upscaling or downstream processing but also achieves an exceptionally high cell concentration in a minimal harvest volume. The latter is crucial for the subsequent transfection of cells using electroporation, which is essential in the production of engineered T cells used in CAR-T and TCR therapies.

Efficient isolation of viable cells from cell cultures is vital for biopharmaceutical production processes, enhancing the quality and efficacy of therapeutic products. In both Ksep® 50 runs, recovery rates were high at 95% in the first run and 91% in the second, ultra-high concentration run. Importantly, the proportion of viable cells—the product of interest—increased to 98% and 96%, respectively, compared to the starting material, proving the outstanding cell sorting capability of the Ksep® 50. In addition, flow cytometric analysis of T cell-specific phenotype markers confirmed that the phenotype was maintained during the Ksep® processing.

Effective downstream processing relies on maximizing throughput to minimize processing time and operating costs. Regardless of the initial cell concentration, both runs demonstrate that approximately 35 L can be processed with one single-use consumable using both Ksep® 50 chambers, though specific process parameters may influence this throughput.

Conclusion

The Ksep® 50 SU counterflow centrifuge enables efficient downstream processing of T cells with high throughput. It achieves exceptional viable cell recovery of 98%, and cell sorting enhances product purity by selectively removing dead cells from the harvest. The high degree of automation and closed system design, including the use of sterile disposables, ensure reliable reproducibility and safety in cGMP-compliant production processes. With processing volumes from 0.1 L to approximately 35 L, Ksep® 50 is the optimal choice for both development and pilot-scale manufacturing. Once parameters are set, production can be seamlessly scaled up to the Ksep® 400, capable of processing bioreactor volumes of up to 200 L.

For more information about Ksep®, visit
www.sartorius.com/ksep

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