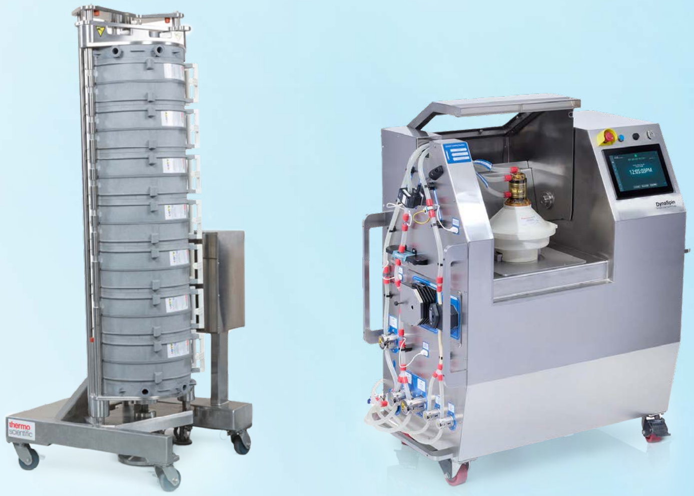
Application note



Harvest and clarification

Single-use clarification across process scales via DynaSpin centrifuges and the Harvest RC clarifier platform

Introduction

In a conventional direct clarification process, a two-stage depth filtration is employed—one stage for primary filtration to remove the bulk cell mass, and a secondary filtration to reach the necessary filtrate quality for downstream purification.

However, as harvest volume and/or cell density increases, the number of depth filters may become logistically intractable due to footprint limitations in the manufacturing suite. Consequently, at volumes >2,000 L, the Thermo Scientific™ DynaSpin™ Single-Use Centrifuge can replace the first stage of depth filtration, directly reducing the number of clarifying filters required [1,2].

Thermo Fisher Scientific now offers a suite of single-use solutions for harvest and clarification of monoclonal antibodies (mAbs)—the Thermo Scientific™ DynaDrive™ Single-Use Bioreactors (S.U.B.s), the DynaSpin™ Single-Use Centrifuge, and a family of clarification products including the Harvest RC Chromatographic Clarifier, Harvest RC Centrate Chromatographic Clarifier, Zeta Plus™ Depth Filter Capsules, and Emphaze™ AEX Hybrid Purifier (Figure 1).

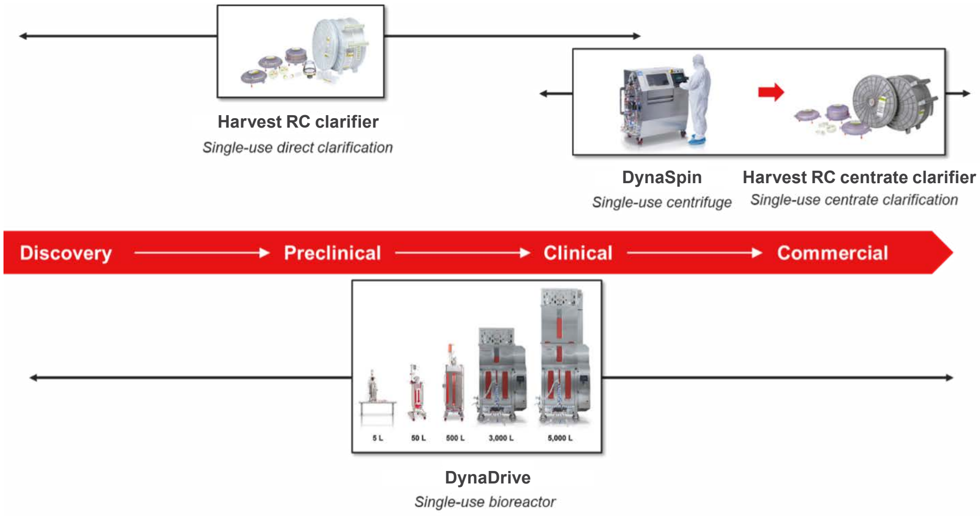


Figure 1. Fully single-use solution for harvest and clarification from lab scale to production.

The DynaSpin centrifuge is a single-use, continuous-flow disk-stack centrifuge designed to streamline harvest operations across scales [3]. Supporting process volumes from 50 L to 5,000 L, it delivers efficient clarification performance, particularly at larger manufacturing scales. The system integrates ergonomic hardware, robust single-use consumables, and intuitive software to simplify operation while reducing both process complexity and facility footprint requirements for the harvest unit operation. The DynaSpin centrifuge enables higher throughput, reduced footprint, and simplified facility design at scale.

The Thermo Scientific™ Harvest RC Chromatographic Clarifier platform comprises the Harvest RC clarifier and Harvest RC centrate clarifier. These solutions provide a high-capacity, synthetic single-use clarification platform that is scalable across all development phases and process volumes for mAb and mAb-like modalities expressed in Chinese hamster ovary (CHO)-derived cell cultures [4]. The solutions in the Harvest RC clarifier platform help provide comparable soluble and insoluble impurity reduction in terms of particle size and DNA clearance. This can simplify downstream process development and technology transfer across internal and external manufacturing footprints [5].

In this study, we evaluated the use of the DynaSpin Single-Use Centrifuge in tandem with the Harvest RC centrate clarifier and obtained high-quality clarified cell culture fluid (CCCF) across a broad range of process conditions selected to demonstrate the robustness of these technologies. Together, the data demonstrate that the combined use of the DynaSpin centrifuge and Harvest RC centrate clarifier offers an opportunity to reduce the cost of goods, footprint, and consumables usage, while providing superior product quality compared to traditional technologies.

Materials and methods

Cell culture and analytics

IgG-producing CHO-K1 cells were thawed, passaged, and expanded into the required process volume, then cultured for 11 days in a fed-batch culture using a combination of either Gibco™ Efficient-Pro™ AGT™ Medium and Efficient-Pro™ AGT™ Feed 1 or ExpiCHO™ Stable Production Medium and EfficientFeed™ C+ AGT™ Supplement. Temperature, pH, and dissolved oxygen (DO) were controlled using online measurements. Viable cell density (VCD) and cell viability were measured using the Beckman Coulter Vi-Cell Blu Cell Viability Analyzer. mAb titer was measured using a Cedex Bio (Roche Custom-Biotech) analyzer.

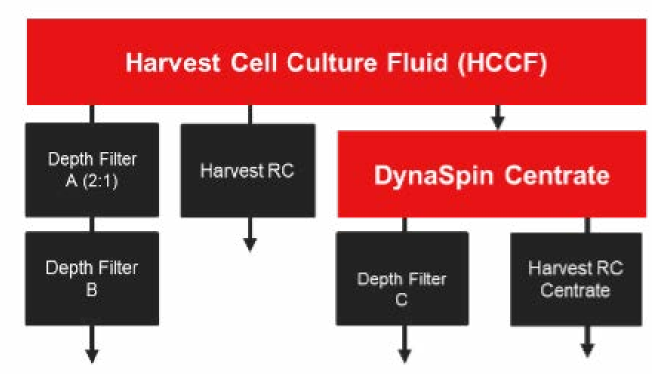
Centrifugation process

Cell cultures were centrifuged using the DynaSpin Single-Use Centrifuge at flow rates of 3.5 L per minute (LPM) or 11 LPM and a rotor speed of 3,600 RPM.

Clarification process

Several lab-scale devices (20–25 cm^2)—depth filters A, B, and C, the Harvest RC clarifier, and the Harvest RC centrate clarifier—were run per standard operating procedures to the designated pressure differential cutoff. Depth filter A was used as the first stage with depth filter B as the second stage, at a 2:1 surface area ratio. As depicted in Figure 2A, the harvested cell culture fluid (HCCF) was processed either by direct clarification (2:1 depth filter scheme or single-stage Harvest RC clarifier) or using a DynaSpin Single-Use Centrifuge. Centrate from the DynaSpin centrifuge was further clarified using either a single-stage depth filter or the Harvest RC centrate clarifier. Clarification flow rates were set at a flux of 100 LMH for the first stage and controlled using peristaltic pumps. The in-line pressure was measured across each device, and the feed and CCCF weights were monitored to calculate volumetric throughputs. After terminal pressure was reached, the devices were flushed to maximize product recovery. Samples were collected for each train and analyzed for turbidity reduction and mAb recovery as described below.

A



B

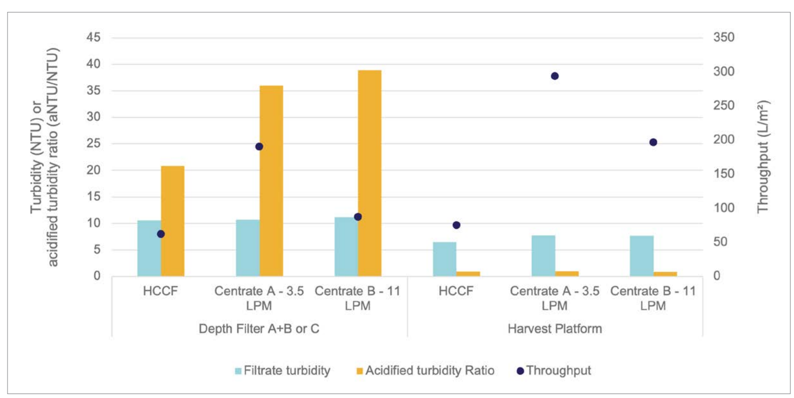


Figure 2. Clarification performance of Harvest RC clarifier platform via direct clarification or DynaSpin centrifuge. (A) Overview of clarification of HCCF through various media. **(B)** Comparison of the quality of CCCF clarified through the various media, measured by turbidity, acidified turbidity ratio, and throughput.

Analytics

Acidified turbidity ratio was used to rapidly evaluate host cell DNA levels. The acidified turbidity ratio is defined as the post acidification turbidity measurement divided by the initial turbidity measurement [6]. Turbidity and acidified turbidity were measured using a Thermo Scientific™ Orion™ AQ4500 Turbidimeter.

Resource modeling

Process models were evaluated using BioSolve Process™ software, version 9.2, from Biopharm Services Ltd (**biopharmservices.com/biosolve-software/biosolve-process**), along with the throughputs generated from experimental data and assuming 5 g/L mAb titer and 5% packed cell volume (PCV). Included in the model are capital charge, consumables, buffer cost, personnel cost, utilities, and waste disposal.

Evaluation of harvest and clarification performance by single-use technologies

The performance of several single-use technologies in clarifying an approximately 5% PCV mAb culture was evaluated. Each train was evaluated for performance based on throughput (L/m^2) and CCCF quality (Figure 2B).

The paired use of the DynaSpin centrifuge and Harvest RC centrate clarifier achieved the highest throughput and the highest-quality CCCF, as measured by low levels of both CCCF turbidity and acidified turbidity ratio, which is correlated with the level of soluble impurities like DNA (see materials and methods). The Harvest RC clarifier and Harvest RC centrate clarifier produced comparable CCCF characteristics from input fluids of HCCF and centrate from the DynaSpin centrifuge, respectively. Specifically, CCCF from either the Harvest RC clarifier or Harvest RC centrate clarifier was characterized by a turbidity <10 NTU, and acidified turbidity ratios were <1 aNTU/NTU. The similar fluid quality can be attributed to the fact that both the Harvest RC clarifier and the Harvest RC centrate clarifier contain quaternary ammonium functionalized fibrous chromatographic media, which captures cell debris and soluble contaminants such as DNA, by electrostatic interactions.

Depth filter A+B and depth filter C produced consistent filtrate turbidity of <15 NTU, but inconsistent acidified turbidity, indicating a breakthrough of soluble contaminants such as host cell DNA. From this dataset, no significant differences in yield were observed—with >95% recovery obtained from all centrate trains.

Importantly, these trends remained even when the centrate feed rate to the DynaSpin centrifuge was increased >3-fold (from 3.5 LPM to 11 LPM). The DynaSpin centrifuge enables flexible operation across a wide range of feed rates, allowing manufacturers to balance throughput and product quality based on process needs. While increasing the flow rate reduces overall processing time, it typically results in decreased separation performance due to lower residence time and higher shear forces, which can increase cell lysis. As expected, when the flow rate was increased from 3.5 LPM to 11 LPM, depth filter C had a >50% reduction in throughput and a decrease in filtrate quality (as observed by the increase in the acidified turbidity ratio from ~35 to ~40 aNTU/NTU). However, the Harvest RC centrate clarifier was still able to maintain consistently high-quality CCCF (<10 NTU and <1 aNTU/NTU) and a throughput reduction of only ~30%.

Next, combinations of cell culture conditions and DynaSpin centrifuge settings were selected that yielded a broad range of centrate fluid qualities, ranging from representative optimized production to highly impure, in order to challenge the DynaSpin centrifuge/ Harvest RC centrate clarifier combination. All the CCCFs from the Harvest RC centrate clarifier, regardless of the purity of the input centrate fluid, were high quality (Figure 3). These results included centrates A and B, which represent a traditional range of centrate turbidities (100–400 NTU), along with centrate C, which would be considered an extremely challenging centrate at ~900 NTU.

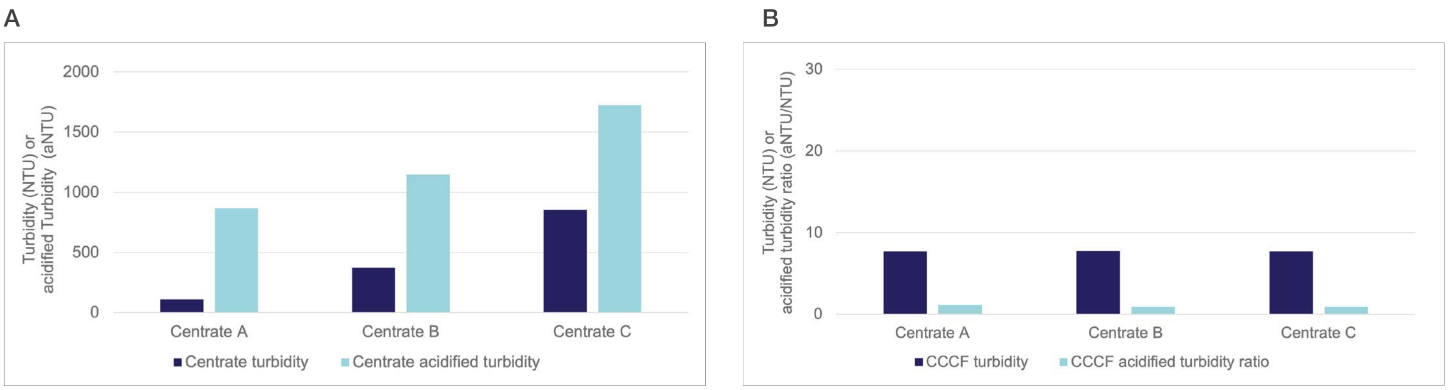


Figure 3. Filtrate quality from Harvest RC centrate clarifier with a range of centrate inputs from the DynaSpin Centrifuge. (A) Comparison of turbidity and acidified turbidity across centrates of varying quality. **(B)** Comparison of CCCF turbidity and acidified turbidity ratios across centrates of varying quality. Each centrate was clarified using the DynaSpin centrifuge and Harvest RC centrate clarifier platform.

Estimated resource and cost implications

BioSolve Process™ software was utilized to evaluate a variety of clarification schemes for their impact on total cost of goods sold (COGS) for 5% PCV cultures at bioreactor scales of 500–5,000 L (Figure 4A). When normalized to a traditional two-stage depth filtration process (black line, Figure 4A), clarification using either the Harvest RC clarifier or the DynaSpin centrifuge and Harvest RC centrate clarifier were modeled to be more cost-effective across all the evaluated bioreactor volumes.

Direct clarification with the Harvest RC clarifier was found to have the lowest COGS up to ~2,000 L, at which point the DynaSpin centrifuge plus the Harvest RC centrate clarifier become more economical. At volumes above ~2,000 L, traditional depth filtration becomes logistically and operationally challenging due to the large number of individual clarifying units and resulting holders, floor space constraints, and increased setup and teardown times. The DynaSpin centrifuge enables a shift in the economic optimum for harvest clarification, becoming the most cost-effective strategy at scales between ~2,000 L and ~5,000 L. The reduction in clarification devices that accompanies the use of the DynaSpin Single-Use Centrifuge and Harvest RC centrate clarifier combination can translate to lower capital investment, reduced facility footprint, and simplified operations.

These pain points are quantifiable drivers behind the lower-COGS switch from direct HCCF clarification to centrifugation followed by centrate clarification (Figure 4B, C). Increased DynaSpin centrifuge feed rate decreased COGS (Figure 4), despite the lower throughputs of clarification devices. As a result, this may provide an opportunity to reduce the number of DynaSpin centrifuges required to process the volume in one shift (Figure 4D). However, since operating at a slower feed rate improves clarity, deploying more DynaSpin units may be preferable. Overall, the combination of the DynaSpin centrifuge and Harvest RC centrate clarifier resulted in up to 59% fewer individual clarification devices, compared to a traditional depth filtration process following a DynaSpin centrifuge step. This decrease in devices can reduce labor, setup time, and supply chain complexity.

The data presented here were modeled on a 5% PCV culture, but for cultures >5% PCV these COGS calculations can be expected to shift in favor of the use of the DynaSpin centrifuge plus Harvest RC centrate clarifier at volumes below 2,000 L, as more individual chromatographic devices are needed to remove the increasing cell mass in direct clarification. Of course, COGS does not always supersede logistical constraints such as facility fit or process simplicity (1-stage vs. 2-stage process). Collectively, these results demonstrate high-quality, fully single-use solutions for harvest and clarification at all considered scales.

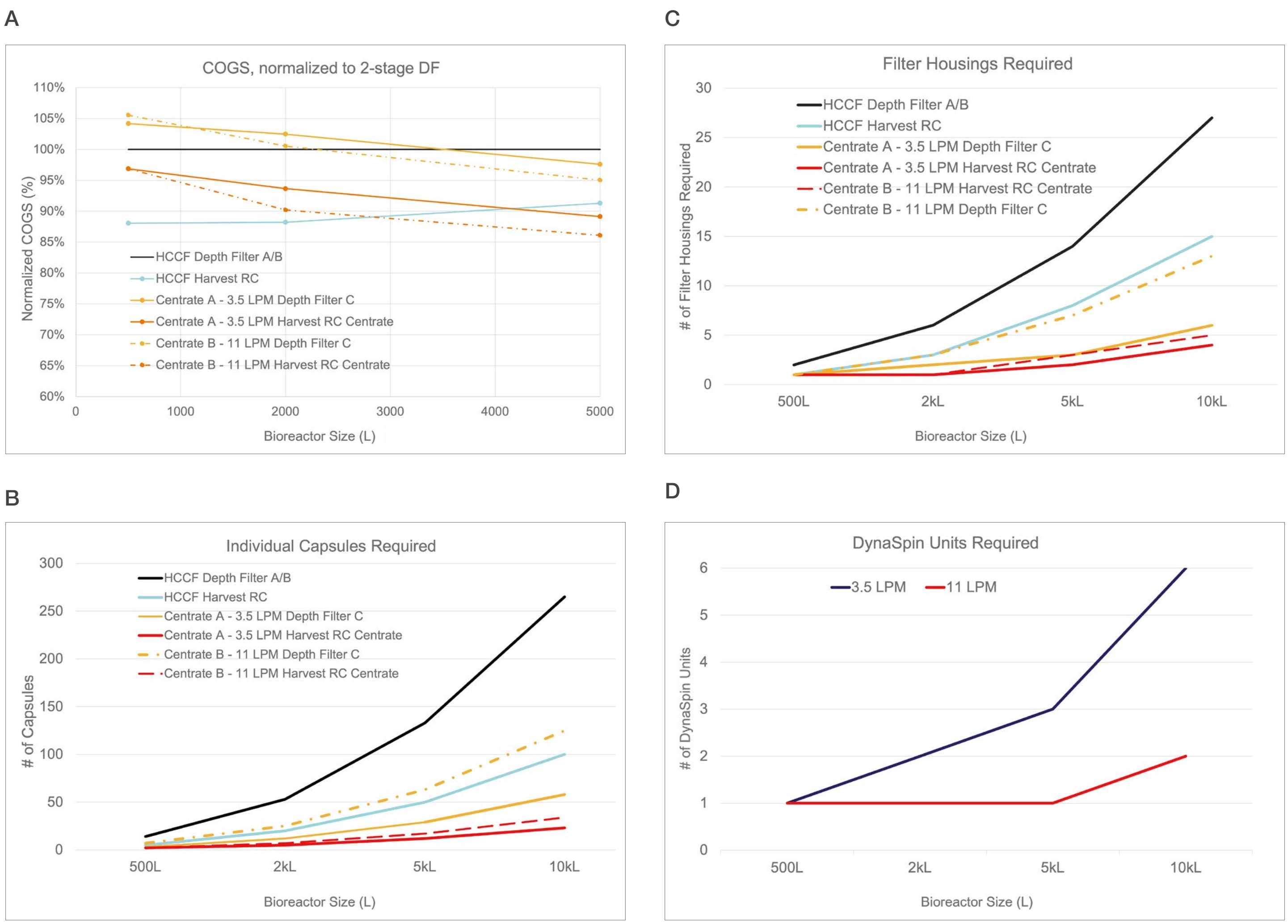


Figure 4. Effect of harvest and clarification on resource requirements and COGS for 500 L to 5,000 L scales. (A) Comparison of COGS associated with various clarifying methods across different sized bioreactors. Normalized to a traditional two-stage depth filtration (DF) process (black line). **(B)** Comparison of number of capsules required for each clarifying method across different sized bioreactors. **(C)** Comparison of number of filter housings required for each clarifying method across different sized bioreactors. **(D)** Comparison of number of DynaSpin units needed for various flow rates across different sized bioreactors.

Discussion and conclusion

In this study, the combination of the DynaSpin centrifuge and Harvest RC centrate clarifier resulted in the lowest surface area requirements and highest CCCF quality. Similar fluid qualities were achieved when the centrate processing time was decreased by ~2/3—giving biopharma manufacturers an opportunity to decrease their processing time at the centrifugation step without compromising the quality of clarification.

The fluid quality and throughput data were used to model a 5,000 L monoclonal antibody bioprocess. In this model, the combination of the DynaSpin centrifuge and Harvest RC centrate clarifier could result in up to 59% fewer clarification devices when compared to the use of traditional depth filtration following the DynaSpin centrifuge step. This reduction in clarification devices can further enable reduction in stock of consumables, warehouse space, labor burden, staging footprint, and waste handling.

The DynaSpin centrifuge enables scalable, high-throughput harvest with reduced infrastructure, while the Harvest RC clarifier platform provides consistent fluid quality. Together, the DynaDrive Single-Use Bioreactors (S.U.B.s), the DynaSpin Single-Use Centrifuge, and the Harvest RC clarifier platform offer a unified, end-to-end single-use harvest platform designed for seamless scale-up, rapid tech transfer, and multi-product manufacturing.

References

1. Next-generation single use harvest solutions: Efficiency for today and sustainability for tomorrow (2023) Thermo Fisher Scientific, Waltham, MA.
2. Spin it first: Finding an optimal harvest solution by considering both cost and sustainability (2023) Thermo Fisher Scientific, Waltham, MA
3. Development and use of a scale-down model with the DynaSpin Single-Use Centrifuge. (2025) Thermo Fisher Scientific, Waltham, MA.
4. Chromatographic capture of cells to achieve single stage clarification in recombinant protein purification (2021) Biotechnology Prog.
5. Sustainability of single use technologies in biopharmaceutical manufacturing (2023) 3M Purification Inc., St. Paul, MN.
6. Enhancing protein A performance in mAb processing: a method to reduce and rapidly evaluate host cell DNA levels during primary clarification. (2019) Biotechnology Prog.

Ordering information

Product		Cat. No.
DynaSpin Single-Use Centrifuge		DSPIN.9000
DynaSpin Single-Use Rotor		SUT00056
Harvest RC Chromatographic Clarifier	BT500	EMP051HRC2FA
	CT15	EMP015HRC2FA
	WP6	EMP006HRC2FA
	BC4	EMP201HRC2FA
	BC25 (25cm^2) Luer	EMP301HRC2FA
	BC25 (25cm^2) Sanitary	EMP303HRC2FA
	BC340	EMP513HRC2FA
	BC1020	EMP533HRC2FA
	BC2300	EMP710HRC2FA
	BC16000	EMP770HRC2FA
Harvest RC Centrate Chromatographic Clarifier	BC4	EMP201RCC020R
	BC25 (25cm^2) Luer	EMP301RCC020R
	BC25 (25cm^2) Sanitary	EMP303RCC020R
	BC170	EMP503RCC020R
	BC340	EMP513RCC020R
	BC1020	EMP533RCC020R
	BC2300	EMP710RCC020R
	BC18400	EMP770RCC020R
Encapsulated System Holder	16EZA 1-High	4552601
	16EZA 2-High	4552603
	16EZA 3-High	4552604
	16EZB	6135601

Abbreviations

aNTU: acidified NTU

CCCF: clarified cell culture fluid

COGS: cost of goods sold

HCCF: harvest cell culture fluid

LMH: liters per m^2 per hour

LPM: liters per minute

NTU: nephelometric turbidity units

RPM: rotations per minute

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