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StreamLink® CC 15: A Novel Downstream Processing System for Ambr® Cell Line Development Workflows

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Abstract

As the global market for biopharmaceutical products continues to expand, there is an ever-growing demand for faster and more efficient ways to deliver new protein drug candidates. Sartorius' Ambr® 15 bioreactor platform is a prominent technology in the cell culture space. Its automation capabilities, high throughput capacity, and scalability make it an ideal tool for producing these new drugs.

The advantages of the Ambr® platform moved the processing bottleneck from the upstream portion of cell line development and screening to the downstream steps of product processing. To mitigate this bottleneck, Sartorius has launched StreamLink® CC 15, an innovative breakthrough in cell line development that will transform how cell culture samples can be clarified and | or purified for downstream analysis. The StreamLink® system is equipped with advanced sensing technology to deliver process control to otherwise uncontrolled downstream processing steps. The combination of Ambr® 15 and StreamLink® CC 15 promises to revolutionize bioprocessing and the speed at which drug candidates can be taken to market. This application note describes the utility of the system in a monoclonal antibody (mAb) production process.

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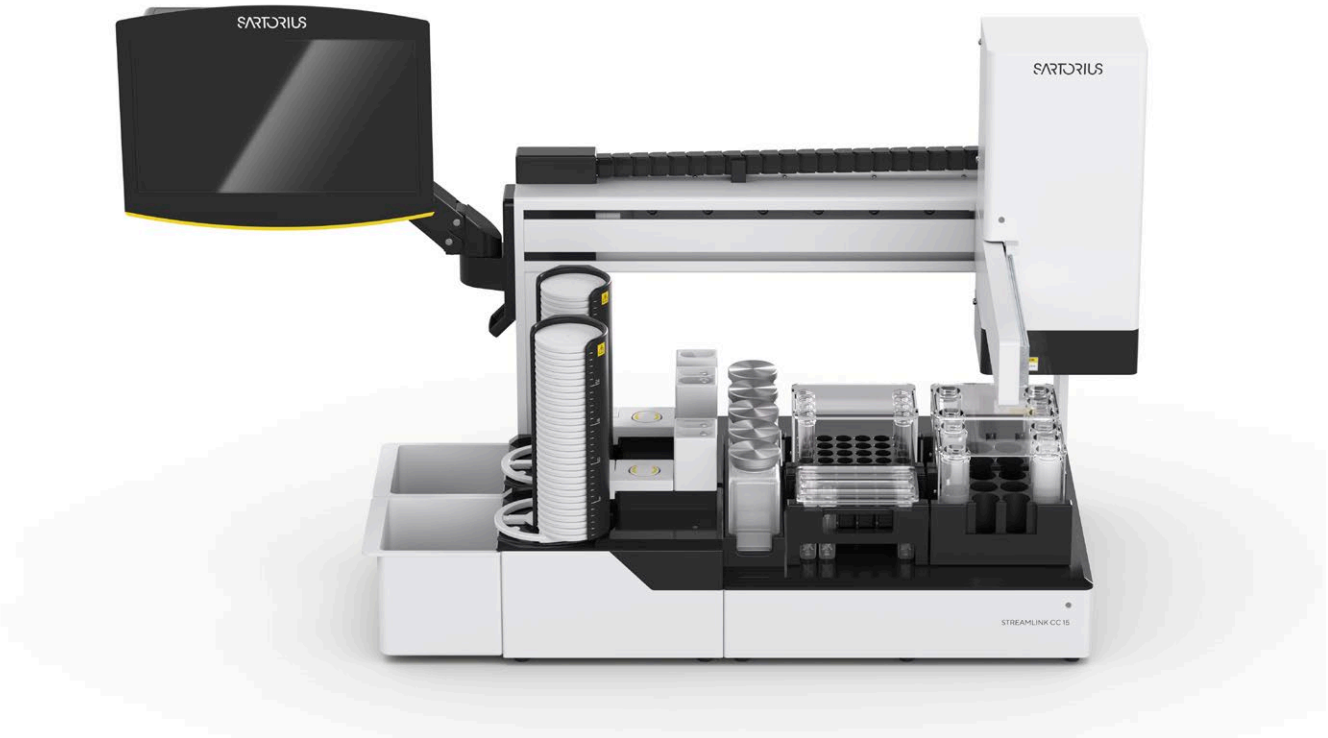
Introduction

Technological advances in early cell line development (CLD) workflows (such as Sartorius' microscale high throughput bioreactor platform, Ambr® 15) have transformed the way early screening for process optimization and clone selection is done within the CLD space.[1–3] Equipped with advanced sensing technologies, the Ambr® 15 allows users to simulate a bioprocessing environment representative of larger-scale bioreactors used in pilot and manufacturing plants. The automation offered by the Ambr® 15 significantly decreases full-time equivalent (FTE) and accelerates CLD timelines due to its high throughput capacity.[1] Despite these advantages, the Ambr® 15 system does not solve downstream processing bottlenecks. Currently, there is no efficient, streamlined solution on the market that performs clarification and purification steps for further analysis of the product of interest.

In general, downstream operators will implement centrifugation or vacuum | syringe-based filtration followed by a chromatography step, which is a labor-intensive approach. In addition, these solutions have the possibility of producing sample-to-sample variation due to their uncontrolled and crude nature, negatively impacting experimental results. To mitigate the bottleneck shifted by Ambr® 15 and to fill a much-needed early CLD workflow demand, Sartorius has developed StreamLink® CC 15, an automated downstream processing solution.[4]

The StreamLink® system can carry out both clarification and purification of 48 mAb-expressing cell culture samples in one setup. The system is fully walkaway with advanced sensing technology and process controls that limit variation between samples[4] to unlock the full potential of the Ambr® 15 workflow. The system also boasts automated error recovery that allows the process to continue even in the event of errors, freeing up operator time for other tasks. In this work, we describe the journey of an Ambr® 15 sample as it gets processed by StreamLink® CC 15 and how the sensing technology and process control allow for consistent and high-quality output.

Figure 1: *StreamLink® CC 15 System*



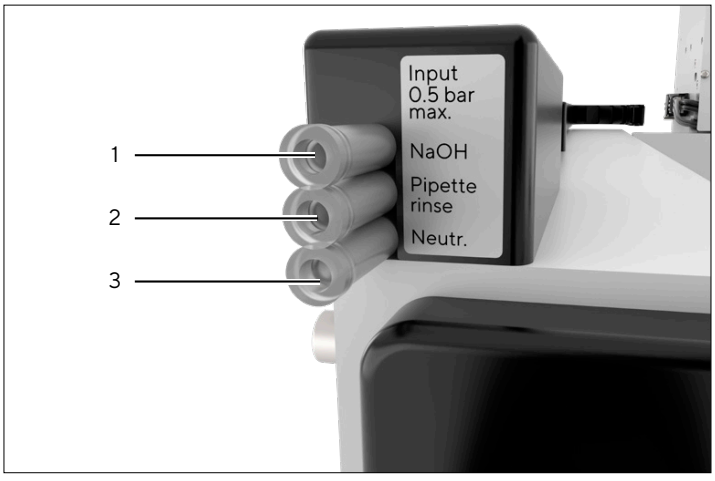
Materials

Hardware

StreamLink® CC 15 is made up of an Ambr® 15 – style liquid handler and two filter stations (Figure 1). The system has been designed such that it can be used inside a biological safety cabinet and on the bench. Each filter station is modular and can function independently of the other. The liquid handler serves each filter station as it delivers input samples from the input labware positions and transfers the outputs to the output labware. In addition, the liquid handler is connected to three liquid lines: NaOH, Pipette Rinse, and Neutralization Buffer (Figure 2). These lines allow the fixed steel tip to be washed at the pipette wash station and deliver a pre-defined volume of neutralization buffer to the output samples if the user enables this function. In terms of liquid lines to the filter station, there are seven valves available: NaOH, Elution, Strip, Wash | Rinse 2, Custom, Equilibration | Rinse 1, and Air (Figure 3).

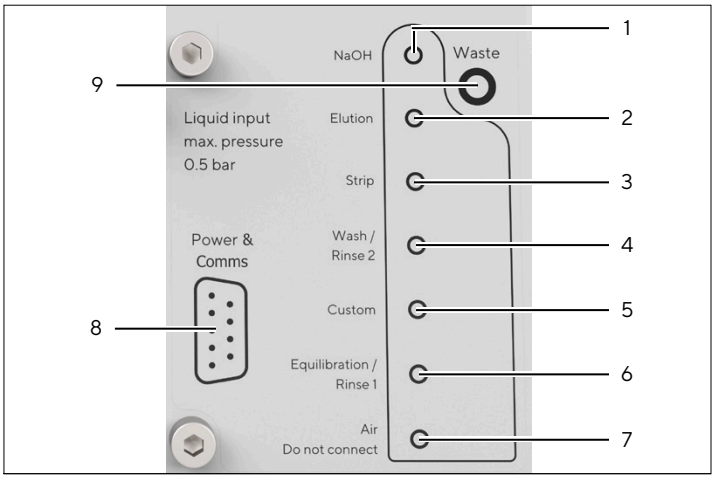
These supply valves allow buffers to be pumped into the filter station during each process as required. The clarification filter clamp (for the Sartoclear® Discs[4] on the filter station) can be closed without a filter to complete the flow path if no clarification is required for the process, i.e., purification-only set ups. Equally, there is a purification device bypass flow path that is used if the system is performing a clarification-only process, as well as during flow- path cleaning in place (CIP) between samples. Purification is done using Sartorius' new Sartobind® Rapid A Nano[4] and is installed on the side of the filter station using a Luer lock clamp system.

Figure 2: *Liquid Lines Connected the Liquid Handler on the StreamLink® System.*



Note. 1 = NaOH, 2 = Pipette Rinse, 3 = Neutralization.

Figure 3: *Liquid Lines and Connections for the Filter Station on the StreamLink® System.*



Note. 1 = NaOH, 2 = Elution, 3 = Strip, 4 = Wash | Rinse 2, 5 = Custom, 6 = Equilibrations | Rinse 1, 7 = Air, 8 = Power and Comms Connection, 9 = Waste Line Connection.

Sensing Technology

StreamLink® CC 15 uses a variety of pressure and liquid sensors to control the movement of samples throughout the flow path. Within the filter station, there are two pressure sensors; one upstream of the Sartoclear® Disc filter and one downstream but still upstream of the Sartobind® Rapid A Nano device, shown in Figure 4 (P1 and P2). These sensors allow for differential pressure PID control across the clarification filter to ensure excess pressure is not exerted upon the sample during clarification, which could compromise its integrity.
In addition to the pressure sensors, the filter station flow path is also fitted with two liquid sensors; one upstream of the peristaltic pump, termed the pre-clarification liquid sensor (Figure 4, A) and another just upstream of the purification device, termed the pre-purification liquid sensor (Figure 4, B).

These sensors allow for the positioning of liquid throughout the flow path as required by the process to ensure that the Sartobind® Rapid A Nano device remains bubble-free, preserving its integrity from cycle to cycle. Downstream of the Sartobind® Rapid A Nano device, the flow path is fitted with a UV sensor which permits the StreamLink® system to give an estimation of the eluted IgG from the sample as well as to give the user the option to use UV peak cutting to concentrate their eluate via defining the AU threshold for elution (Figure 5).

Within the liquid handler, a liquid sensor allows liquid detection during aspiration and dispensing. An inline pH probe gives a reading every time liquid is aspirated or dispensed to allow the purified output samples to be neutralized (Figure 6).

Figure 4: *Schematic of the StreamLink® CC 15 System Filter Station Flow Path.*

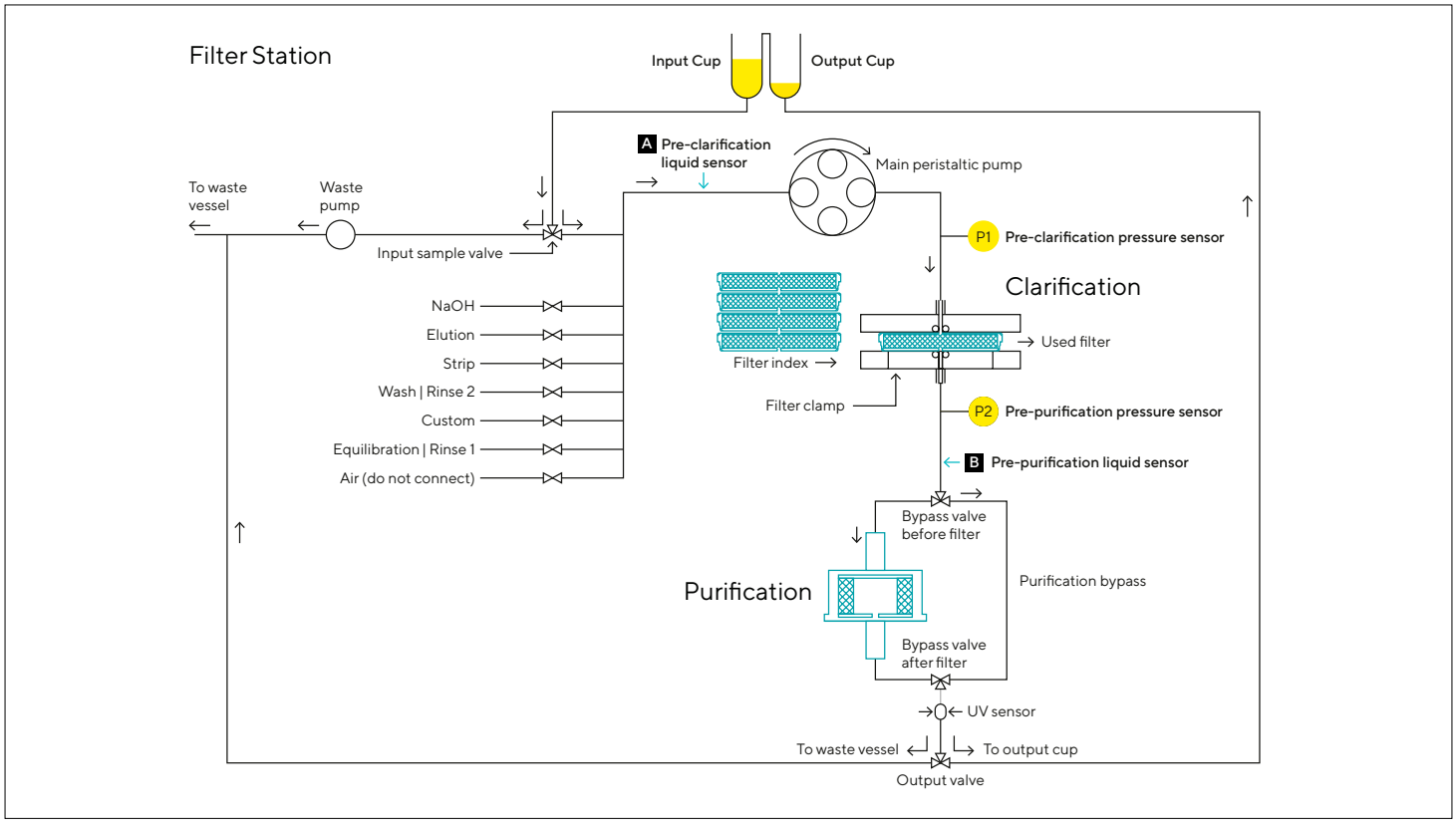
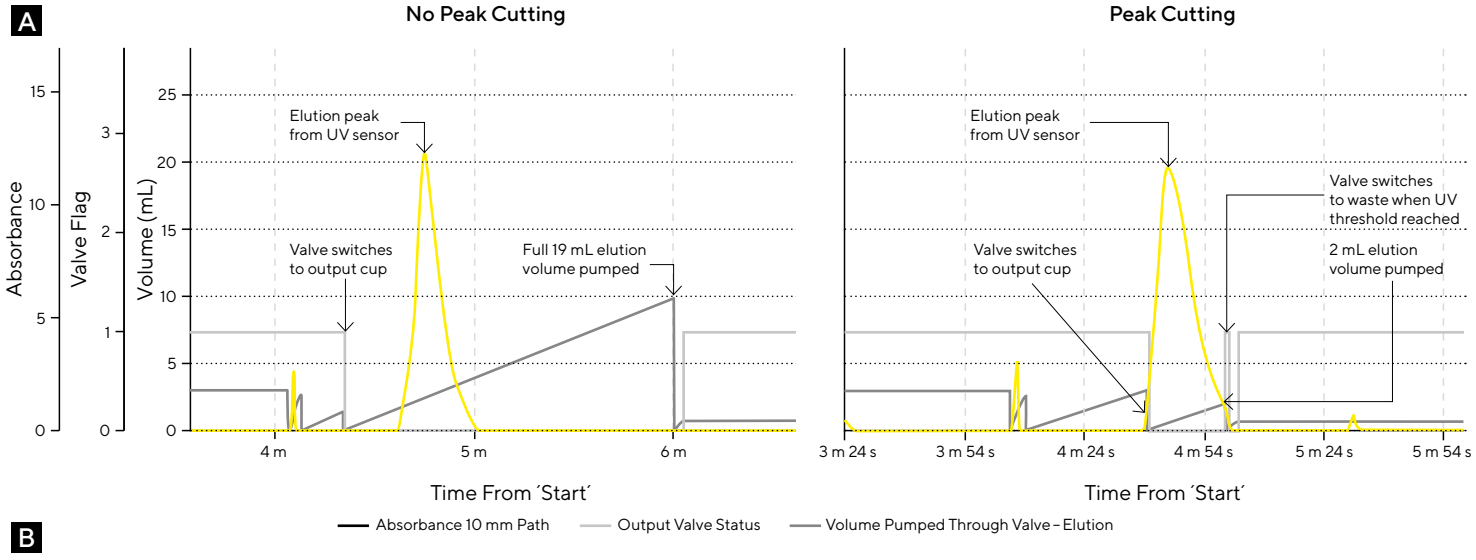
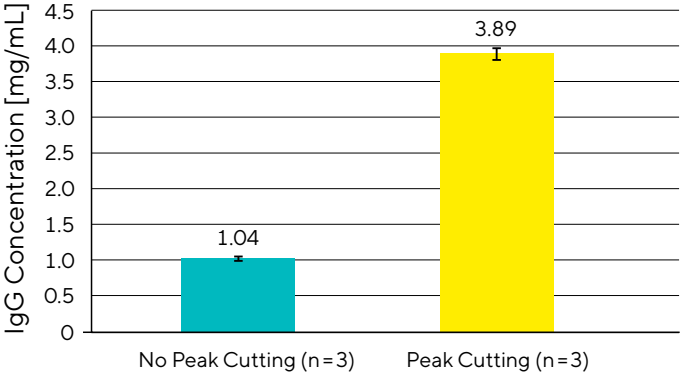


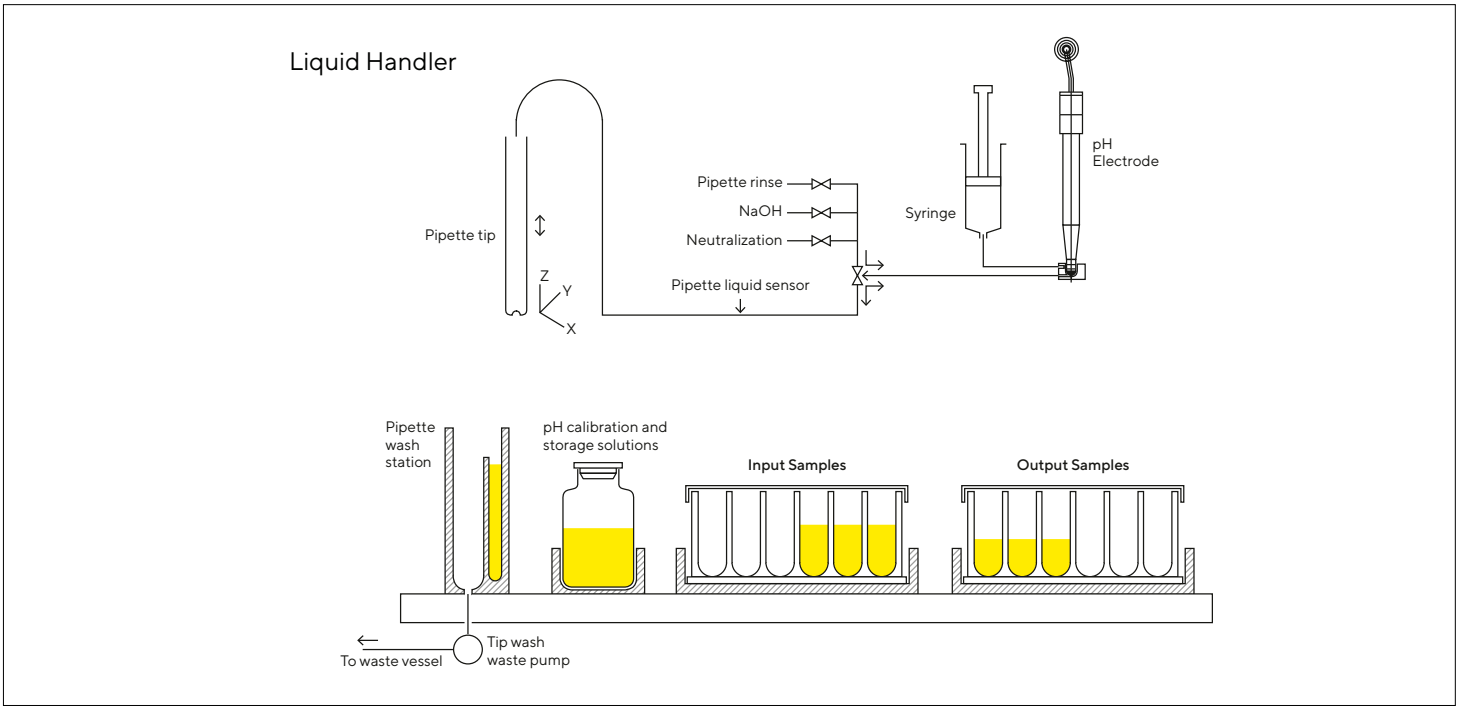
Figure 5A and B: *System-Generated Data Showing the Valve States and Pumped Volumes During the Elution With and Without Peak Cutting (A). Eluate Concentrations of the Same Initial IgG Load With and Without Peak Cutting (B).*





Note. UV absorbance signal during elution is also shown.

Figure 6: *Schematic of the StreamLink® CC 15 System Liquid Handler Flow Path and Bed.*



Methods

Process Workflows

The StreamLink® CC 15 system is designed to carry out three main processes in downstream cell culture workflows: clarification, purification, or a combined process. Each of these processes can be modified through the template creation wizard to suit each user's specific processing needs. Variables such as volumes and flow rates can be adjusted, and advanced features – such as the use of second clarification filters or UV peak cutting to concentrate eluate – can be enabled. A schematic workflow of each of these processes is shown in Figure 7. Each process workflow is preceded by a system start-up process where critical self-checks, calibrations, and a full system CIP are performed. Once the chosen process workflow is complete (clarification, purification, or combined), the system will automatically go through the end-of-run process where there is a full-system CIP followed by the system storing itself in the user's chosen storage solution, such as PBS | EtOH.

Table 1 shows the buffers connected to the system, how each buffer line is used during the processes as well as any alternative names they may have in the template creation wizard.

Figure 7: *Schematic Workflow of Processes That Can Be Done With the StreamLink® CC 15 System.*

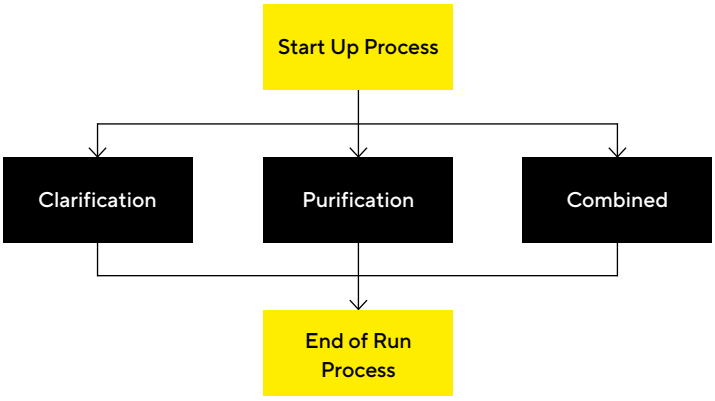


Table 1: *Nomenclature of Liquid Lines | Buffers Used in the StreamLink® CC 15 Processes and Hardware.*

Filter Station Valve	Alternative Name	Use During Processing
NaOH	-	CIP
Elution	-	Eluting IgG from the Sartobind® Rapid A Nano post-sample washing steps
Strip	-	Optionally stripping the Sartobind® Rapid A Nano device post-elution as part of the CIP
Wash | Rinse 2	Pre-wash buffer	Washing of the Sartobind® Rapid A Nano device post-sample binding to remove contaminants such as DNA
Equilibration | Rinse 1	Equilibration buffer or Rinse buffer	1. Rinsing through the clarification filter, post-sample clarification 2. Equilibrating the Sartobind® Rapid A Nano device prior to sample binding and post-CIP 3. Washing of the Sartobind® Rapid A Nano device post-binding (and pre-wash if enabled)

Results

Clarification

Process Overview

For clarification processes, users can directly load their Ambr® 15 vessels from their fed-batch process onto the StreamLink® CC 15 sample deck. The user simply needs to uncap the Ambr® 15 vessels and place them in the Ambr® 15 labware vessel holder for the StreamLink® CC 15 before starting the process.

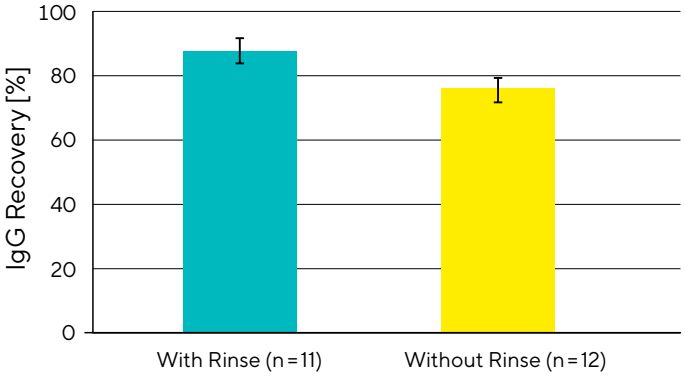
Once the process is initiated, the StreamLink® CC 15 software will automatically prompt the user to complete the necessary start-up activities (such as loading the correct amount of clarification filters). This eliminates the need for time-consuming and monotonous buffer and consumables calculations. When the user required start-up activities are complete, the system goes through an automated start up procedure to ensure it is ready to take the first sample (Figure 7).

Once completed, the liquid handler goes to the Ambr® 15 vessel rack to collect the first sample and dispenses it into the input cup of the filter station that the software has assigned for that sample (Figure 8A). From here, the filter station's peristaltic pump will push the sample through the Sartoclear® Disc filter that has been loaded into the filter station to process the sample (Figure 8B). As the sample is being clarified, proportional integral derivative (PID) control ensures excess pressure is not exerted on the sample using two pressure sensors in the flow path: one upstream of the clarification filter and one downstream to allow differential pressure control (Figure 4). Once the whole sample has been pushed through the filter, the system will pump a rinse buffer through the filter (if this option is selected in the template) to ensure that all the sample is pushed through (Figure 8C), maximizing sample recovery (Figure 9).

During clarification, the flow path valve status is open to the Sartobind® Rapid A Nano bypass (since purification is not being carried out) so that once the sample is pushed through the clarification filter, it goes directly to the output cup following an air push through the filter to get any remaining sample out.

From here, the liquid handler will collect the clarified sample from the filter station output cup and dispense it into the specified output labware on the system bed (Figure 8D) while the filter station goes through a CIP procedure before the next sample (Figure 8E). This process is repeated on both filter stations until all the samples are processed.

Figure 9: *Recovery of IgG Using the StreamLink® CC 15 System for a Clarification Run.*



Note. 5 mL of rinse buffer was used in the "With Rinse" condition. For the "With Rinse" group, n=11. For the "Without Rinse" group, n=12.

Error Recovery and Process Control During Clarification

The Sartoclear® Disc filters are rated to fully clarify culture samples of up to 40 million cells/mL. However, sometimes filter blockages can still occur. In the event of a filter over-pressure | blocking error during clarification, the system will discard the remaining sample, and the liquid handler will collect only the sample which passed through the filter before blocking occurred. This is a common error in filtration workflows, and the sample will usually need to be recovered manually or discarded by the user. With StreamLink® CC 15, the user can select an optional second filter feature in the clarification template. This will allow the system to predict when the filter will block and automatically take a new filter to process any remaining sample. If filter rinse is enabled, the system will push the rinse buffer through the filter before taking a second filter to maximize sample recovery (Figure 9). With this feature, the system can automatically recover and process the entirety of the sample, so the user does not need to manually intervene in the event of a filter-blocking error.

Figure 8: *Workflow of a Clarification Process for StreamLink® CC 15.*

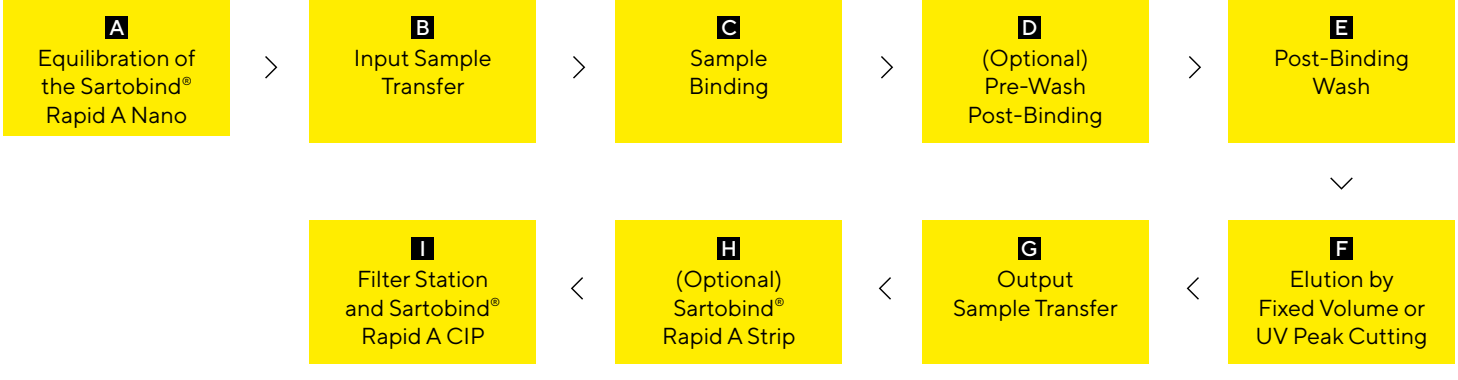
Purification

Process Overview
As with the clarification process, the StreamLink® CC 15 software will prompt the user to complete the necessary start-up tasks before the process can begin. For purification, this can also include reminding the user to exchange the Sartobind® Rapid A Nano for a new one if required. This can be done through the system maintenance panel in the software. Once everything is in place, the system will begin its automated start-up procedure before starting the purification process (Figure 7).

Following start-up, the filter stations will begin to pump equilibration buffer through the purification device to ensure the Sartobind® Rapid A Nano filter is at a neutral pH prior to sample loading (Figure 10A). The liquid handler will then collect the first sample from the input labware and take it to the input cup (Figure 10B), from which the filter station will begin pumping it into the Sartobind® Rapid A Nano device to bind the IgG (Figure 10C). Liquid sensors upstream of the Sartobind® Rapid A Nano (Figure 4) will ensure that only sample or buffers enter the device and prevent air from entering. Following binding, the system will begin to pump wash | equilibration buffer(s) through the device to remove any contaminants or unbound proteins from the Sartobind® Rapid A Nano (Figure 10D and E).

Within the purification template, the user can define whether they want to include a pre-wash buffer (Figure 10D) as part of this stage in the process or the default singular wash buffer (Figure 10E). Following the wash stages, the process can either elute the bound IgG using a fixed volume defined in the template, or use UV peak cutting via the UV sensor downstream of the Sartobind® Rapid A Nano device to concentrate the eluate with a user-defined UV absorbance threshold (Figure 10F). Regardless of the elution option, the sample will end up in the filter station output cup, where the liquid handler will collect and dispense it into the output labware (Figure 10G). During this, the filter station will begin a CIP process to prepare for the next sample, which can include an optional strip of the Sartobind® Rapid A Nano device with a low pH buffer to remove any remaining IgG that may still be bound to the ligand (Figure 10H and I). During the strip, the UV absorbance will be tracked in the flow path, which will highlight to the user any uneluted or contaminant proteins still left in the device following the elution step.

Figure 10: *Workflow of a Purification Process for the StreamLink® CC 15.*



Error Recovery and Process Control in Purification

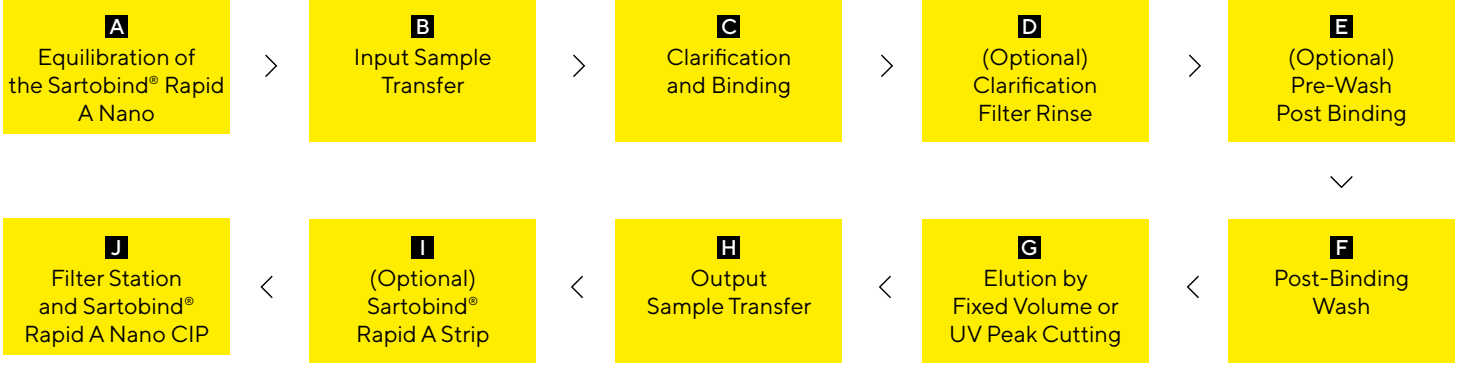
As the cycle number of the Sartobind® Rapid A Nano device increases, the performance is likely to degrade due to the ligand's exposure to NaOH during CIP. With StreamLink® CC 15, Sartobind® Rapid A Nano filter health can be monitored from run to run using backpressure. During a purification process, the pressure sensors in the filter station flow path will track the back pressure of the Sartobind® Rapid A Nano device. Increased backpressure from the device will activate PID control to slow down the pump so that excess pressure is not exerted on the sample. This decrease in flow rate will be highlighted on the run screen in the software and the end of run report as deviations. If the user frequently sees these prompts indicating a decrease in flow rate due to high pressure, it may be a sign that the Sartobind® Rapid A Nano ligand health has degraded, and the binding capacity has potentially decreased. The process will automatically prompt the user to run an extensive maintenance CIP on the device or replace it if high back pressures are seen during sample processing. As a result, the user does not have to worry about losing sample due to unknowingly using a degraded Sartobind® Rapid A Nano.

Combined

Process Overview

The combined process combines the clarification and purification processes into a singular workflow. Following the start-up procedure, the process begins as a standard clarification where the liquid handler will collect the sample from the input labware and dispense it into the filter station input cup for processing through the clarification filter (Figure 11B). However, unlike a clarification-only process, the Sartobind® Rapid A Nano is simultaneously equilibrated (Figure 11A) with the flow path valve states open to the device instead of the bypass. This means that once the sample is clarified through the Sartoclear® Disc filter, it goes directly into the pre-equilibrated Sartobind® Rapid A Nano device to bind the IgG (Figure 11C). If clarification filter rinse is enabled in the template, this will also pass directly from the clarification filters into the device (Figure 11D). Once the product is bound, the clarification filter is ejected, the flow path is closed and rinsed from any remaining cells via the Sartobind® Rapid A Nano bypass. From here, the process follows the purification-only workflow where the product, bound to the ligand, undergoes washing steps (including pre-wash if enabled) (Figure 11E and F), followed by elution (either by fixed volume or peak cutting) (Figure 11G) before being collected from the filter station output cup by the liquid handler and taken to the output labware (Figure 11H). As with all processes, the filter station and the liquid handler then undergo a CIP procedure (which can include the optional strip of the Sartobind® Rapid A Nano device) before handling the next sample (Figure 11I-J).

Figure 11: *Workflow of a Combined Process Using the StreamLink® CC 15.*



Discussion

High-throughput, automated bioreactor systems like the Ambr® 15 create more efficient and robust CLD workflows. However, elevated productivity in upstream activities places an increased burden on downstream steps, requiring innovative solutions to clarify and purify harvested material. This application note showcases how the StreamLink® CC 15 solves the processing bottleneck created by Ambr® 15 workflows, offering a solution to accelerate CLD activities and reduce time-to-clinic.

The StreamLink® CC 15 has three configurable processes (clarification, purification, and combined processing), meaning it has the flexibility to operate in various process setups. The combined processing power eliminates the need for multiple clarification and purification systems, creating a smaller footprint and simplifying downstream operations. Additionally, the easy-to-use template creation wizard is intuitive and user-friendly, requiring less employee training and specialization.

The system is complemented by sensing technologies that offer enhanced process control, including pressure sensors to track differential pressure across the clarification filter, liquid sensors to ensure no bubbles enter the Sartobind® Rapid A Nano device, and UV sensors that enable peak cutting and estimation of product concentrations.

Finally, just as the Ambr® 15 represents a small-scale model for larger bioreactors, the StreamLink® CC 15 is a scaled-down version of Sartobind® devices, speeding up the transition from process development to large-scale production.

Conclusion

The StreamLink® CC 15 reduces FTE and accelerates CLD workflows by speeding up clarification and purification steps. The system is designed to seamlessly integrate into existing products within our CLD portfolio, creating a simplified, integrated workflow.

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