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# Combining Ambr® 15 With Streamlink® CC 15 Facilitates Efficient Clone Selection and Enables High-Throughput Clarification and Purification Processes

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## Abstract

This application note highlights the capabilities of the Ambr® 15 bioreactor system and the StreamLink® CC 15 high-throughput automated clarification and purification system, and demonstrates their potential to facilitate clone selection. As demand for cost-effective and time-efficient bioprocessing increases, combining Ambr® 15 with StreamLink® CC 15 offers a powerful solution to high-throughput automated workflows from cell cultivation to clarification and purification. We also detail the benefits of using StreamLink® CC 15 for efficient cell culture clarification and Protein A purification.

Combining Ambr® 15 with StreamLink® CC 15 can help scientists streamline early-stage development, making it a compelling choice for biopharmaceutical companies looking to streamline clone selection and purification in order to accelerate biotherapeutic development.

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# Introduction

Biotherapeutics are rising in popularity due to their potential to treat a range of diseases including certain cancers, autoimmune disorders, and chronic conditions. To keep up with increasing production, there is a growing demand for time-efficient and cost-effective bioprocessing methods. Current methods of clone selection, clarification, and purification are often inefficient, requiring frequent operator intervention that can delay development.

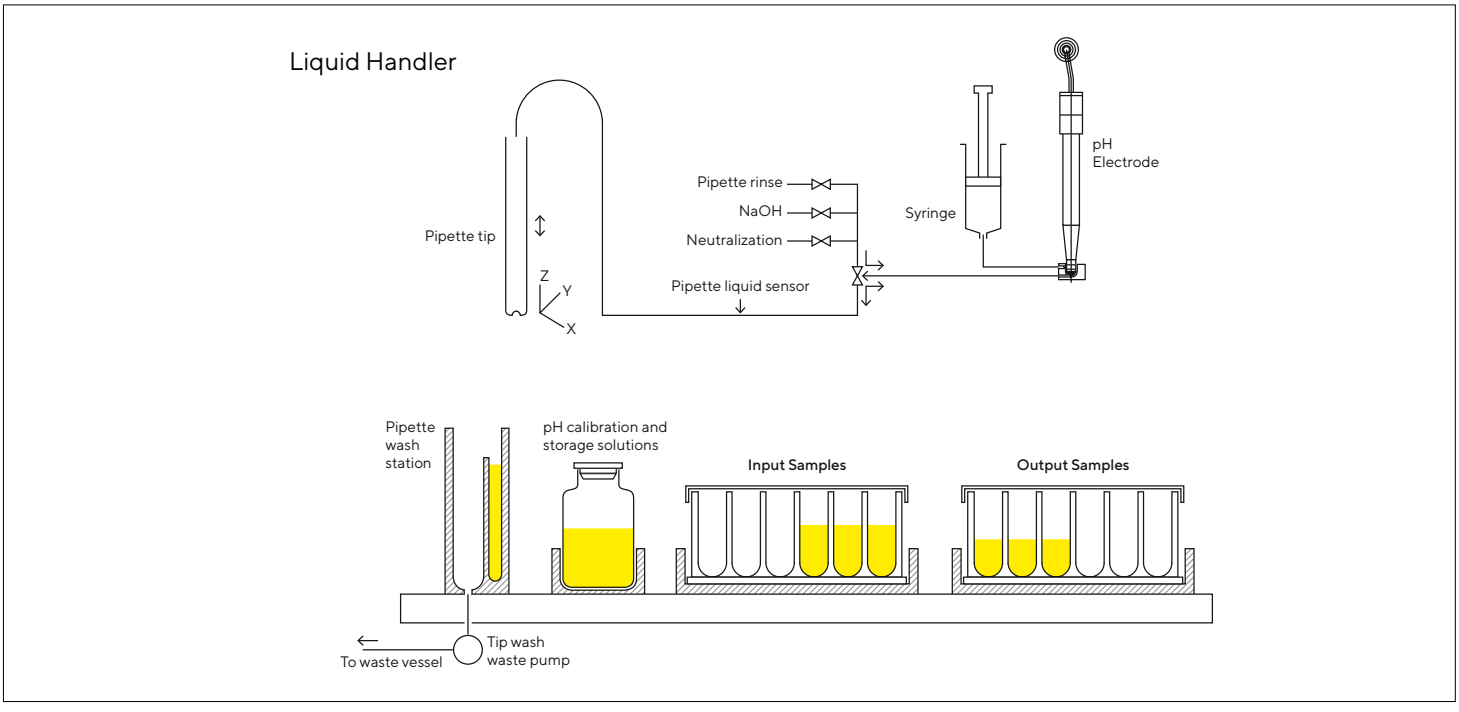
This application note compares current methods with a combination of new systems showing potential to accelerate and streamline cell line selection. We discuss data obtained from a cell line development workflow using Ambr® 15 and StreamLink® CC 15 systems. We performed purification of an IgG1 molecule using integrated clarification and protein A (PrA) capture chromatography in the StreamLink® CC15 system. This automated purification was performed using StreamLink® CC 15 and compared with an alternative in-house method. For both purification methods, we determined recoveries and quality attributes of the IgG1 molecule.

## StreamLink® CC 15 System

StreamLink® CC 15 from Sartorius is a fully automated high-throughput system designed for rapid clarification and purification of low-volume (5 – 15 mL) cell cultures. The system accelerates and automates sample preparation, targeting a throughput of 24 – 48 samples per experiment, to improve the bottleneck in the clone selection process and expedite product quality feedback. StreamLink® CC 15 was designed with easy-to-use and simplified method creation and a fully automated process to help operators save time.

The system's hardware includes one liquid handler and two filter stations. The liquid handler performs the automated transfer of samples in and out of the two filter stations using a syringe pump with an integrated pH flow cell (Figure 1).

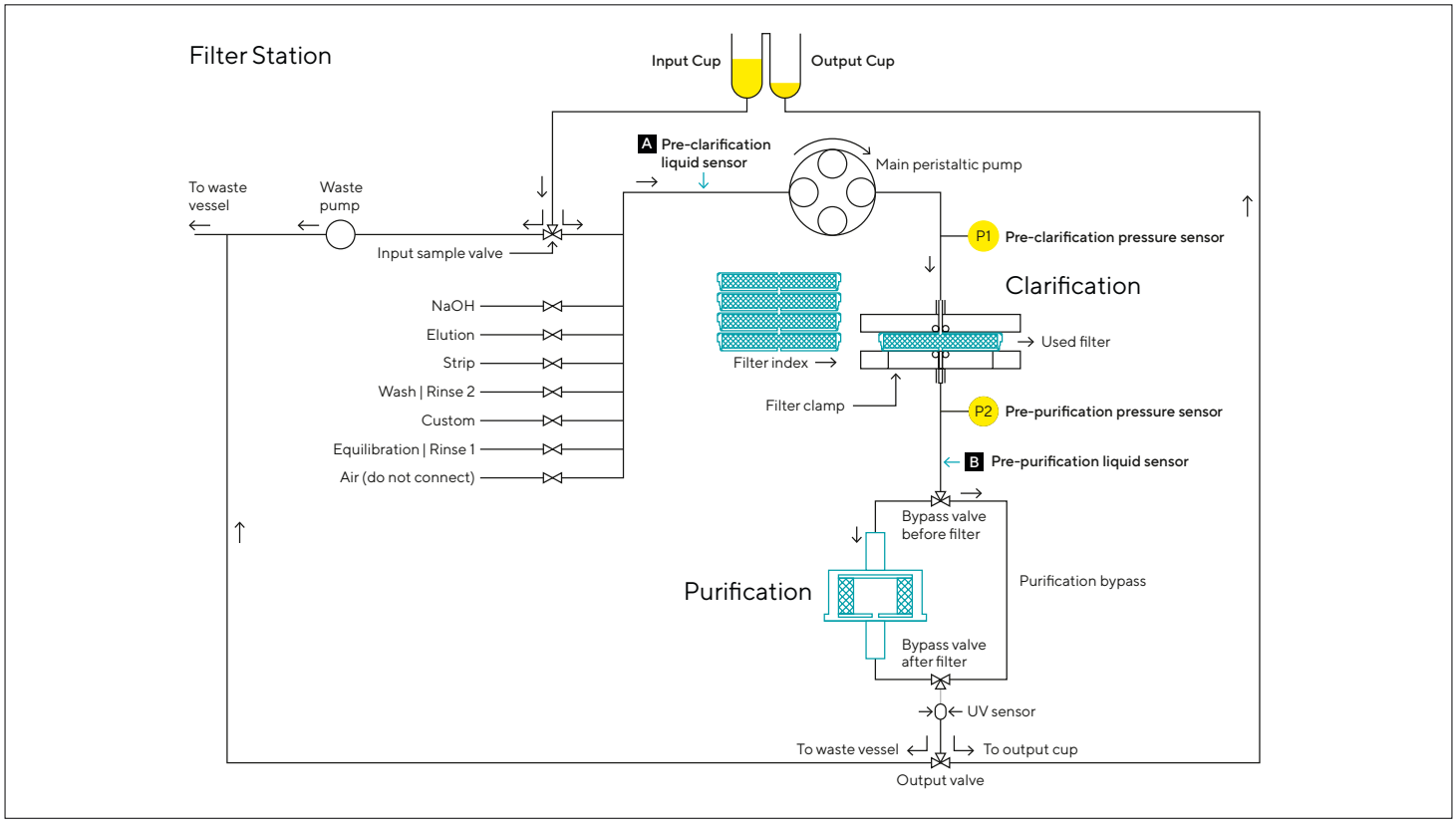
**Figure 1:** *Schematic of the StreamLink® CC 15 System Liquid Handler Flow Path and Bed*



The filter stations process the samples through either the Sartoclear® Disc for clarification or the Sartobind® Rapid A Nano for purification, or both. A peristaltic pump moves the sample and any of the connected process liquids through the filter stations as needed (Figure 2). StreamLink® CC 15 software automatically coordinates the valves, pressure sensors, liquid sensors, and UV detector to complete clarification and purification according to user-defined conditions.

In addition to fully automated and controlled sample preparation, StreamLink® CC 15 can neutralize the eluted product and aliquot output samples, further automating the sample preparation workflow.

**Figure 2:** *Schematic of the StreamLink® CC 15 System Filter Station Flow Path.*



# Materials and Methods

## Ambr 15® Fed Batch

Using the Ambr® 15 bioreactor system from Sartorius, we cultured 48 monoclonal CHO cell lines producing an IgG1 antibody in fed-batch (FB) operational mode. The sparged vessels were operated in FB mode for 14 days in using standard process conditions. The clones were cultured in a custom medium supplemented with 4 mM L-glutamine and 1:1,000 anti-clumping agent (ACA). From day 3, the cultures were fed with 2/0.2% (v/v) Feed A/B and 20% glucose solution, targeting a goal of 4 g $L^{-1}$ glucose level each day. The vessels were inoculated in pre-warmed and supplemented medium at a target cell density of $0.5 \times 10^6$ viable cell (vc) $mL^{-1}$.

We analyzed the cultures daily for viable cell density, viability, average cell diameter, glucose, lactate, $NH_4^+$, $pO_2$, $pCO_2$, glutamine, glutamate, and various ion concentrations. We also monitored pH with the integrated Nova Biomedical® Bioprofile® FLEX2 Automated Cell Culture Analyzer. During the run, we monitored pH, dissolved oxygen (DO), temperature, gas flow, and agitation rates online using Ambr® 15 software. DO concentration was controlled at 40% air saturation. The stirring speed was set to a constant 1,200 rpm. Nitrogen gas flow rate was set to a constant value of 0.2 mL $min^{-1}$, and the oxygen flow rate was controlled and increased by the DO cascade controller depending on oxygen demand. pH was controlled at a set point of 7.05 with a deadband of 0.05, with two-way pH control utilizing $CO_2$ gas and 1 M sodium carbonate solution.

The operating conditions are summarized in Table 1.

On days 8, 10, 13, and 14, we removed 0.1 mL of sample and centrifuged (5 min at 300 g) to remove cell debris before storing the supernatant at -20 °C for titer determination with Octet® RED96e using protein A biosensors. On day 14, we harvested the cultures and transferred them for clarification and Protein A purification using StreamLink® CC 15.

**Table 1:** *Operational Parameters of Ambr® 15 Vessels*

| Operational Parameter | Setpoint \| Information | Unit | Notes |
|---|---|---|---|
| Culture vessel | Ambr® 15 | N/A | N/A |
| Inoculation density | $0.5 \times 10^6$ | vc $mL^{-1}$ | N/A |
| Post-inoculation volume | 15 | mL | N/A |
| Temperature | 37 | °C | N/A |
| pH setpoint | 7.05 | N/A | Two-way control using $CO_2$ gas and 1 M sodium carbonate base solution |
| pH deadband | 0.05 | N/A | N/A |
| DO setpoint | 40 | % | Control loops using $N_2$ and $O_2$ gases |
| Agitation | 1,200 | rpm | Down-pumping, constant |
| Impeller | 1 | No. of units | Stretched blade, diameter 11.4 mm |
| Impeller power number | 2.15 | N/A | Determined by Sartorius |
| Flow rate (macrosparger) | Cascade-dependent | mL $min^{-1}$ | Increased depending on oxygen demand |
| Sparger type | Macrosparger tube | N/A | N/A |
| Headspace aeration | N/A | N/A | No headspace aeration |
| Antifoam addition | 20 | µL $day^{-1}$ | From a 3% stock solution |

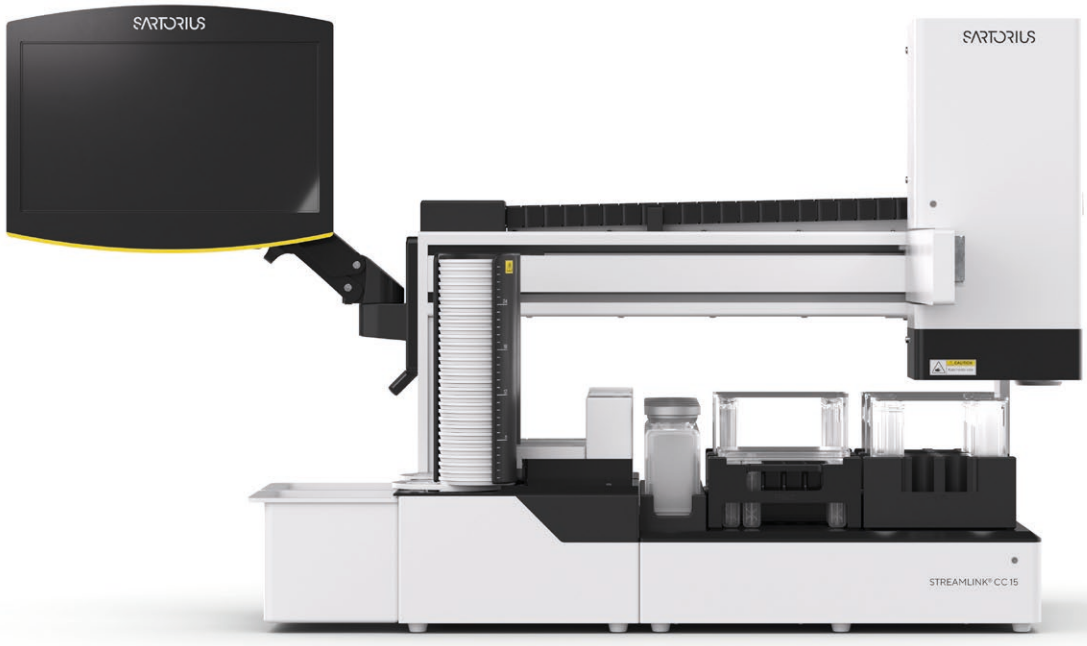
## StreamLink® CC 15

We selected 21 IgG1-producing CHO cell lines to pass on to the StreamLink® CC 15 system. This selection included clones with varying levels of viability and peak viable cell density (VCD), ensuring a diverse and heterogeneous group of clones. StreamLink® CC 15 clarified the harvest (5 mL) from selected clones using single-use Sartoclear® Disc (0.2-µm depth filtration membrane) with an effective surface filtration area of 20 cm². Using PrA affinity chromatography, we purified the clarified cell culture fluid (CCCF) using Sartobind® Rapid A Nano membrane (1.2 mL) technology. Table 2 shows the chromatographic conditions. For all 21 CCCFs, 5.0 mL was loaded on a Sartobind® Rapid A Nano membrane. Elution peak cutting was performed from 100 mAU in the ascending part of the peak to 100 mAU in the descending part of the peak.

**Table 2:** *Chromatographic Method for Purification of IgG1 Using Sartobind® Rapid A Nano Membrane Technology*

| Chromatographic Step | Buffer | Membrane Volumes [MV] | Flow rate [mL/min] |
|---|---|---|---|
| Equilibration | 20 mM NaPi + 150 mM NaCl, pH 7.0 | 10 | 12 |
| Wash 1 | 40 mM NaAc, pH 5.5 | 15 | 12 |
| Sample load | CCCF | 4.2 | 6 |
| Elution | 40 mM NaAc, pH 3.5 | 3.33 | 6 |
| Strip | 0.5 M HAc | 10 | 12 |
| Sanitization | 0.2 M NaOH | 10 | 6 |
| Post-CIP flush | 20 mM NaPi + 150 mM NaCl, pH 7.0 | 10 | 12 |

To compare critical quality attributes (CQAs) of the IgG1 molecule, the 21 selected IgG1-producing CHO clones were simultaneously clarified using centrifugation and purified by an alternative PrA plate purification method. Of the purified IgG1, we tested the following CQAs: glycan profiling, fragmentation quantification by capillary electrophoresis (CE-SDS), and charge variant distribution by cation exchange chromatography (HPLC-CEX).

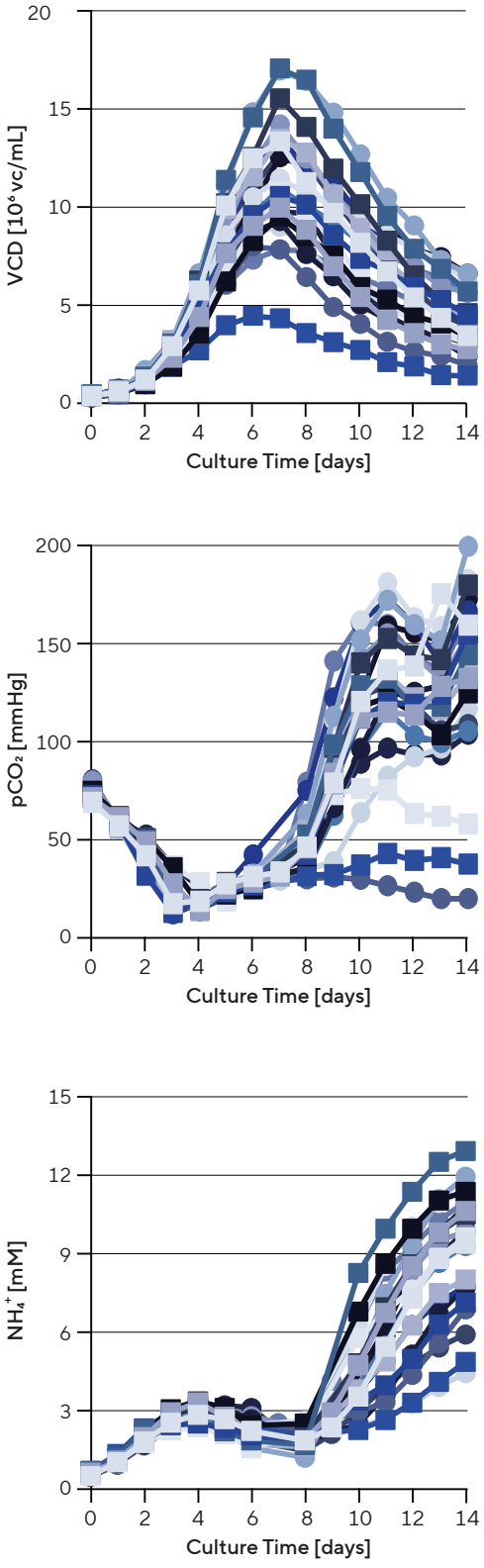


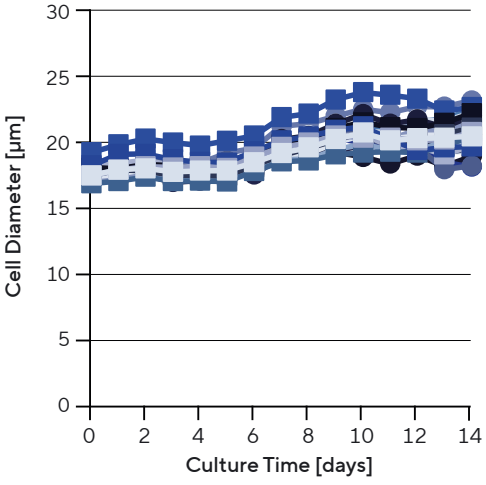
# Results

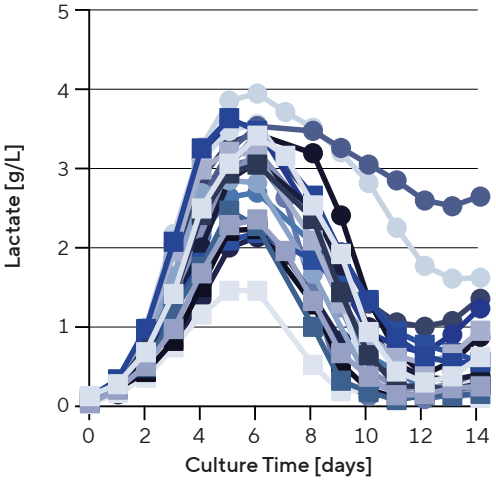
## Ambr 15® Fed Batch

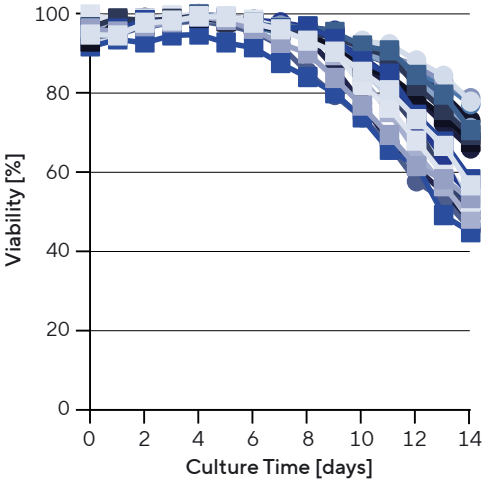
Figure 3 depicts the growth, metabolite, and titer data of the Ambr® 15 – 48 run. Peak viable cell density (VCD) of the clones ranged from 4.5 – 17.1 × $10^6$ vc $mL^{-1}$. The viability of the clones decreased as a function of time to a m inimum of 45% on day 14 (cl. 216). Cell diameter shows differences between the clones. However, the general trend is similar between clones for this variable; it is increasing (or stagnant) as a function of time. Glucose was controlled at 4 g $L^{-1}$ of target glucose level. Lactate concentration increased up to 3.9 g/L on day 5. The lactate profile shows similarities in lactate production for all clones before day 6. The consumption of lactate starts on Day 6, causing a decrease in lactate concentration until the end of the cultivation. Ammonium ion concentration profiles during the run show similarities. This variable increases up to 13.1 mM on day 14 (cl. 305). Additionally, the $pCO_2$ levels show heterogeneity between clones, ranging from 23.0 – 199.0 mmHg. The titer of the selected clones ranged from 0.6 – 2.8 g/L. The highest producer was clone 303.

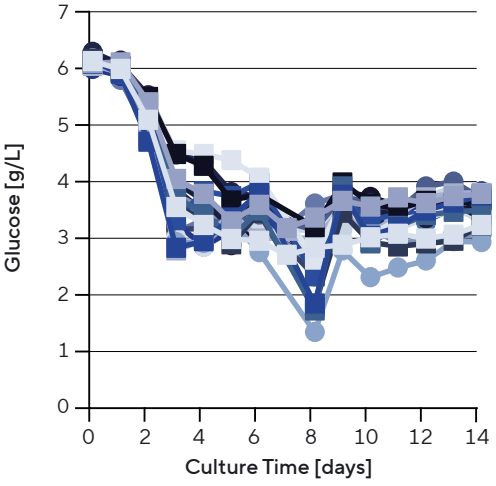
**Figure 3:** *Ambr® 15 Fed-Batch Growth, Metabolite, and Titer Results*

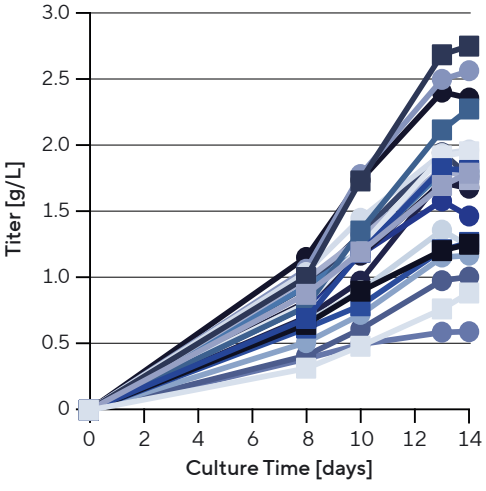


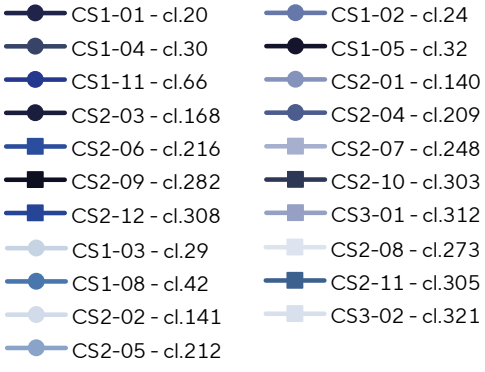

Cell Diameter [µm]
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14
Culture Time [days]


Lactate [g/L]
5
4
3
2
1
0
0 2 4 6 8 10 12 14
Culture Time [days]


Viability [%]
100
80
60
40
20
0
0 2 4 6 8 10 12 14
Culture Time [days]


Glucose [g/L]
7
6
5
4
3
2
1
0
0 2 4 6 8 10 12 14
Culture Time [days]


Titer [g/L]
3.0
2.5
2.0
1.5
1.0
0.5
0
0 2 4 6 8 10 12 14
Culture Time [days]


CS1-01 - cl.20
CS1-02 - cl.24
CS1-04 - cl.30
CS1-05 - cl.32
CS1-11 - cl.66
CS2-01 - cl.140
CS2-03 - cl.168
CS2-04 - cl.209
CS2-06 - cl.216
CS2-07 - cl.248
CS2-09 - cl.282
CS2-10 - cl.303
CS2-12 - cl.308
CS3-01 - cl.312
CS1-03 - cl.29
CS2-08 - cl.273
CS1-08 - cl.42
CS2-11 - cl.305
CS2-02 - cl.141
CS3-02 - cl.321
CS2-05 - cl.212

# StreamLink® CC 15: Purification Performance for Clone Screening

For each of the 21 selected CHO cell lines, 5 mL of CCCF was loaded on the Sartobind® Rapid A membrane. Membrane load varied as a result of the different titer per clone. Membrane loads were between 0.5 and 2.9 mg of IgG1 per mL of PrA membrane. The eluate volume ranged from 5.2 to 7.6 mL and the concentration of the PrA purified clones ranged from 0.32 to 1.31 mg/mL.

Table 3 shows the recoveries obtained for the 21 loaded amounts of IgG1.

**Table 3:** *Obtained Recoveries of IgG1 on StreamLink® CC 15*

| Clone # | Recovery of IgG1 [%] | Clone # | Recovery of IgG1 [%] |
|---|---|---|---|
| 20 | 67.5 | 29 | 62.5 |
| 32 | 60.0 | 66 | 68.0 |
| 141 | 67.3 | 209 | 66.7 |
| 216 | 48.5 | 273 | 73.7 |
| 303 | 73.0 | 308 | 68.3 |
| 321 | 64.1 | 30 | 70.0 |
| 24 | 61.7 | 140 | 67.3 |
| 42 | 68.3 | 212 | 71.2 |
| 168 | 67.5 | 282 | 68.8 |
| 248 | 70.5 | 312 | 71.6 |
| 305 | 68.1 | | |

We determined the CQAs for the IgG1 of the 21 purified eluates Figure 4 compares the results of glycan profiling on clone 303, purified by StreamLink® CC 15 and compared to an alternative purification method.

When comparing purification with StreamLink® CC 15 with the alternative purification method, we observe no significant differences in the glycan profile of IgG1. Figure 5 contains a comparison of charge variant distribution obtained with both purification methods.

Figure 5 shows that, for all purified clones, the obtained charge distribution profile is very similar between the two purification approaches. The average standard variation among clones was 0.8%, 2.1%, and 1.4% for total basic species, main peak, and total acidic species, respectively.

Figure 6 contains an overview the fragmentation profile comparison. The fragmentation profiles between StreamLink® CC 15 and the alternative purification method are very similar, with the exception of clone 305.

**Figure 4:** *Glycan Profile of Clone 303 Purified by StreamLink® CC 15 (Red Line) Compared to an Alternative Purification Method (Blue Integrated Peaks).*

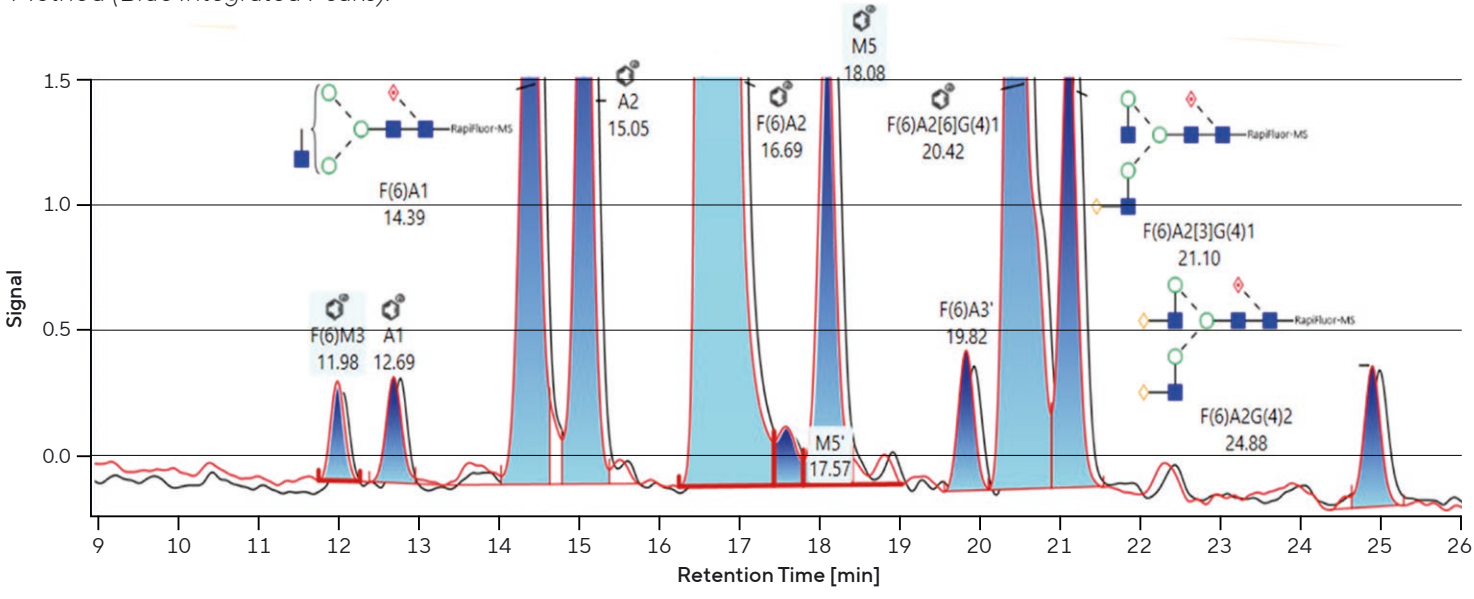


**Figure 5:** *Cex-Hplc Charge Distribution Profiles of Streamlink® CC 15 and Alternative Purification Method: (**A**) Acidic, (**B**) Main, (**C**) Basic Species.*

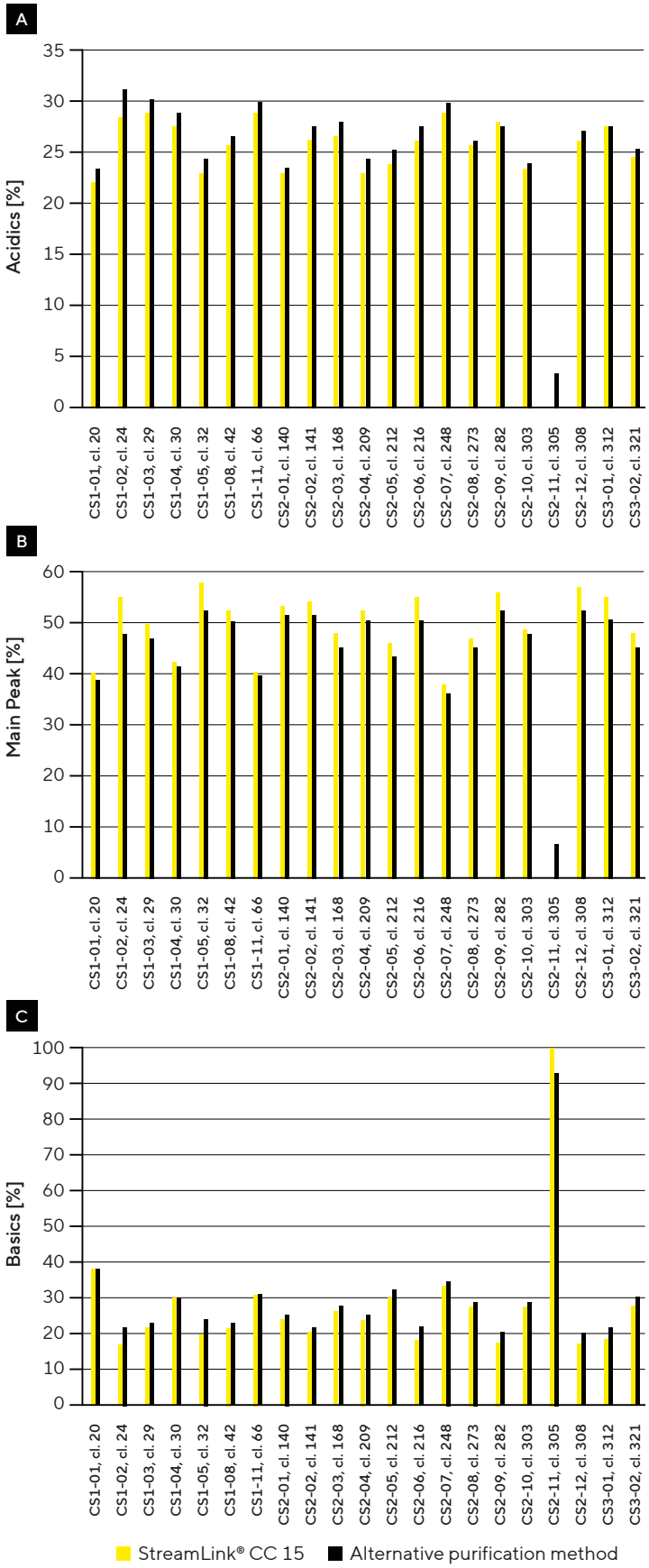
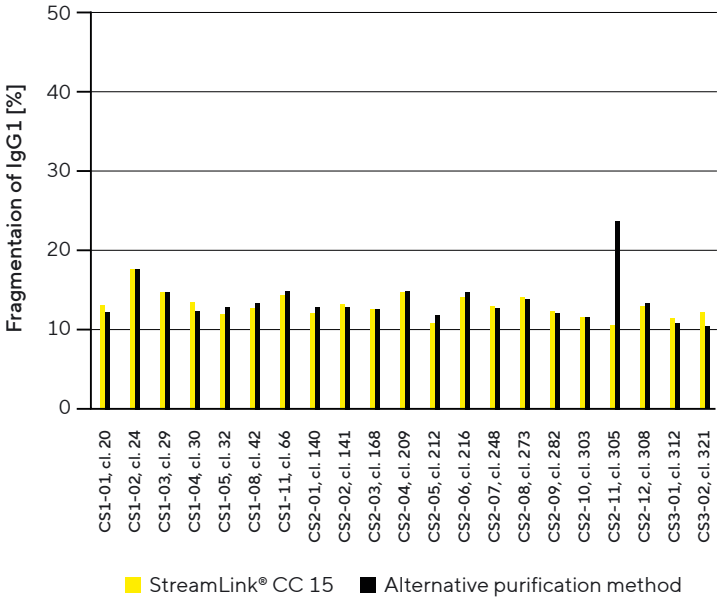



**Figure 6:** *CE-SDS Fragmentation Profile Comparison Streamlink® CC 15 and Alternative Purification Method.*



# Conclusion

Implementing an integrated workflow that combines Ambr® 15 and StreamLink® CC 15 for clone selection provides an efficient process for early-stage development. Obtained CQAs for IgG1 align well with the tested alternative purification method. We obtained recoveries between 48.5% and 73.7% with a non-optimized StreamLink® CC 15 method — with optimization, results could potentially reach >90% recovery.

StreamLink® CC 15 provides similar data to conventional methods for clarification and purification with a 60% reduction in operator time, lowering the risk of human error and increasing overall efficiency of the process. The StreamLink® CC 15 real-time sensing data enables users to remotely monitor processes online, and potentially glean deeper insights than existing workflows can provide. Altogether, these benefits can help streamline clone selection and reduce the time needed to obtain critical product quality feedback.

The increasing popularity of biotherapeutics is leading to rising demand for time-efficient and cost-effective bioprocessing solutions. By combining these systems, manufacturers can progress from development to production more rapidly, and ultimately accelerate the timeline for biotherapeutics.

# References

Z. Reynolds et. al, StreamLink® CC 15 – Novel Sequential High-Throughput Clarification and Purification of Monoclonal Antibodies for Cell Line Development. Sartorius & GSK Application Note, 2022.

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